PARASITOLOGY

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CONTENTS

Nos. 1, 2 (January 1947)

PAGE

Mer, G., Birnbaum, D. and Aioub, A. The attraction of mosquitoes by human beings. Bachi, R. Statistical analysis of data 1

Baylis, H. A. The larval stages of the Nematode *Mermis nigrescens*. (With 11 Figures in the Text) 10

Turk, Frank A. Studies of acari. IV. A review of the lung mites of snakes. (With 9 Figures in the Text) 17

Milne, A. The ecology of the sheep tick, *Ixodes ricinus* L. Some further aspects of activity, seasonal and diurnal. (With 3 Figures in the Text) 27

Milne, A. The ecology of the sheep tick, *Ixodes ricinus* L. The infestations of hill sheep. (With 6 Figures in the Text) 34

Satchell, G. H. The larvae of the British species of *Psychoda* (Diptera: Psychodidae). (With 84 Figures in the Text) 51

Webb, J. E. The structure of the cuticle in *Eomenacanthus stramineus* (Nitzsch), (Mallophaga). (With Plate 1) 70

Edwards, E. E. and Arthur, D. R. The seasonal activity of the tick, *Ixodes ricinus* L., in Wales. (With 12 Figures in the Text) 72

Nagaty, H. F. *Dipetalonema evansi* (Lewis, 1882) and its microfilaria from *Camelus dromedarius*. (With 11 Figures in the Text) 86

Davies, T. I. and Rees, Gwendolen. *Andrepigynotaenia haematopodis*, n.g., n.sp., a new protogynous tapeworm from the oystercatcher *Haematopus ostralegus occidentalis* Neumann. (With 17 Figures in the Text) 93

Crofton, H. D. The second ecdysis of *Trichostrongylus retortaeformis* (Zeder). (With 1 Figure in the Text) 101

Akhtar, S. A. A new genus of Nematodes, parasitic in the pika. (With 11 Figures in the Text) 104

Muspratt, J. Note on a ciliate protozoon, probably *Glaucoma pyriformis*, parasitic in culicine mosquito larvae 107

No. 3 (July 1947)

Feldman-Muhsam, B. Resistance of larvae and nymphs of *Hyalomma savignyi* Gerv. to various conditions of temperature and humidity. (With 2 Figures in the Text) . . 111

Stephenson, William. Physiological and histochemical observations on the adult liver fluke, *Fasciola hepatica* L. I. Survival *in vitro*. (With 1 Figure in the Text) . . . 116

Stephenson, William. Physiological and histochemical observations on the adult liver fluke. *Fasciola hepatica* L. II. Feeding. (With 1 Figure in the Text) 123

PAGE

STEPHENSON, WILLIAM. Physiological and histochemical observations on the adult liver fluke, *Fasciola hepatica* L. III. Egg-shell formation. (With Plate II and 4 Figures in the Text) 128

STEPHENSON, WILLIAM. Physiological and histochemical observations on the adult liver fluke, *Fasciola hepatica* L. IV. The excretory system. (With 3 Figures in the Text) . . 140

JÍROVEC, O., SCHÄFERNA, K. and ŠKORPIL F. *Sporozoon tincae*, a pathogenic parasite of the tench. (With Plates III–V, containing Figs. 1–33) 145

SIMMONDS, F. J. The biology of *Phytodietus pulcherrimus* (Cress.) (Ichneumonidae, Tryphoninae) parasitic of *Loxostege sticticalis* L. in North America. (With 16 Figures in the Text) 150

STEWARD, J. S. Host-parasite specificity in coccidia: infection of the chicken with the turkey coccidium, *Eimeria meleagridis* 157

BAYLIS, H. A. A redescription of *Uncinaria lucasi* Stiles, a hookworm of seals. (With 6 Figures in the Text) 160

BISHOP, ANN, BIRKETT, BETTY and GILCHRIST, BARBARA M. The response of blood-inoculated and sporozoite-induced infections of *Plasmodium relictum* to drugs . . 163

SMYTH, J. D. Studies on tapeworm physiology. II. Cultivation and development of *Ligula intestinalis* in vitro. (With Plates VI–VIII and 4 Figures in the Text) 173

No. 4 (FEBRUARY 1948)

HOBSON, A. D. The physiology and cultivation in artificial media of nematodes parasitic in the alimentary tract of animals 183

REES, GWENDOLEN. A study of the effect of light, temperature and salinity on the emergence of *Cercaria purpurae* Lebour from *Nucella lapillus* (L.). (With 13 Figures in the Text) . 228

TURK, FRANK A. On two new species of tick. (With 11 Figures in the Text) . . . 243

HEISCH, R. B. and GARNHAM, P. C. C. The transmission of *Spirochaeta duttoni* Novy & Knapp by *Pediculus humanus corporis* de Geer 247

SHARIF, M. Nutritional requirements of flea larvae, and their bearing on the specific distribution and host preferences of the three Indian species of *Xenopsylla* (Siphonaptera) . 253

SHUTE, P. G. and MARYON, M. The gametocytocidal action of paludrine upon infections of *Plasmodium falciparum* 264

128
140
145
150
157
160
163
173
183
228
243
247
253
264

VOLUME 38, NOS. 1 & 2 JANUARY 1947

THE ATTRACTION OF MOSQUITOES BY HUMAN BEINGS

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STATISTICAL ANALYSIS OF DATA

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THE PROBLEM

The female *Anopheles* feeds on warm-blooded animals which it finds in the open or in buildings. Observations on the behaviour of these mosquitoes in the open lead to the conclusion that it is not by mere chance that they find their source of food. Something directs the mosquitoes from their breeding place towards the abode of the prey and enables them to retain their sense of direction while flying around and choosing the place and time of attack.

The nature of these directive and attractive forces is unknown. It was studied by van Thiel (1935, 1937) and Reuter (1936) but no decisive conclusions were arrived at. It was assumed that the factors inducing the mosquitoes to feed and those attracting them towards the source of food were identical; humidity, temperature, CO_2, are mentioned among the factors involved.

The problem, in our view, can be divided into three phases: the attraction of *Anopheles* to the human or animal abodes, the attraction of the host, and finally the stimulus to feed.

The present study deals with the second aspect, the attraction of *A. elutus* females from a certain limited distance by human beings.

EXPERIMENTAL SET-UP

A quadrangular cage screened on all sides, 180 cm. long, 60 cm. wide and 60 cm. high was used. Wire-netting screens were inserted inside the cage, 20 cm. from each end. The screens were fixed to the roof of the cage and could be raised and lowered as required. By lowering these screens to the floor of the cage, two compartments 20 cm. long were formed, one at each end of the cage; these compartments will be referred to below as the 'end-compartments'.

The 'bait' was placed opposite one end of the cage at a distance of 30 cm. from it and at the same horizontal level. In experiments where an electric fan was used to produce an air current directed towards the cage, the fan was placed so that the fan, the 'bait' and the centre of the end compartment were in one line—the long axis of the cage.

Six experimental series were arranged with the cage in a *horizontal* position:

(1) In the first series (consisting of twelve tests) an adult was placed in front of the cage, facing the front compartment at a distance of 30 cm. Head, neck and upper part of the chest were on a level with the cage.

(2) In the second series the set-up remained the same but the subject was placed with his back to the front compartment.

(3) In the third series the 'bait' was placed in front of the cage, but the air he expired was led out of the room through a rubber tube.

(4) To check the results of the preceding series of experiments, the air expired by a person outside the room was conveyed through a rubber tube 5 metres long into the experimental room and directed towards the front compartment of the cage through a glass funnel attached to the tube and placed at a distance of 30 cm. from the cage.

(5) In this series of experiments the arrangement was the same as in series no. 3. The expired air was led outside, but an electric fan placed 1 m. behind the seated person created an air current directed towards the cage. The fan was also in action during the preliminary period, before the subject was placed in the experimental position.

(6) In this series the person was standing with his back towards the cage at a distance of 1·5 m. from it, keeping well outside the air current. During the period of exposure, only his left arm was exposed to the current of air directed towards the cage. As in the preceding experiment, the fan was in action also during the preliminary period.

Two more series of experiments were done, in which the cage was suspended from the ceiling in a *vertical* position so that the end compartment faced downward and the bait was placed below it.

(7) In this series the subject was sitting directly underneath, his head being 30 cm. below the cage.

(8) In this series the experiment was arranged in the same fashion except that the air expired by the person was led away from the cage by a rubber tube.

The actual procedure of the experiment was as follows: A given number of hungry *A. elutus* females were introduced into the cage and allowed half an hour to settle down.

Prior to the actual experiment, two countings of mosquitoes were made with the dividing screens closed, one 10 min., and one immediately before the experiment was started.

The end compartment containing the lowest number of mosquitoes was used for the experiment, the 'bait' being placed in front of it.* After 15 min. during which the mosquitoes were allowed to fly freely in all compartments of the cage, the screens were again closed in order to ascertain the effect, if any, of the attraction exerted by the bait—this attraction being expected to cause an increase in the number of mosquitoes in the compartment nearest the bait.

In the first series of experiments, the mosquitoes in the compartment at the opposite end of the cage were also counted, prior to and after the experimental period, in order to ascertain a possible decrease in the number of mosquitoes it contained, owing to the attraction of the human bait at the opposite end.

RESULTS OF THE EXPERIMENTS

Detailed data concerning each of the experiments in the eight series described above are given in Table 1, Appendix I. Their main results may be summarized and interpreted as follows:

1. *The proportion of mosquitoes in the end compartment before exposure to the stimulus*

The total length of the cage was 180 cm., that of the two end compartments together 40 cm. Therefore, the percentage of mosquitoes in the two end compartments in the event of equal and uniform distribution in all parts of the cage should have been about $40/180 = 22{\cdot}22\,\%$.

Actually in the twelve experiments of the first series where the percentage of mosquitoes in both the end compartments was known this percentage in the two compartments was much higher than expected ($56{\cdot}18\,\%$, of which $84/566 = 14{\cdot}84\,\%$ were in the end compartment with the lesser number of mosquitoes, and $234/566 = 41{\cdot}34\,\%$ in the opposite compartment).

It appears, therefore, that the mosquitoes did not distribute themselves equally in the various sections of the cage, but had a definite tendency to prefer one or other of the end compartments to the central compartment.

The cause—or causes—of this preference for the end compartments is unknown. It seems that the manner in which the mosquitoes were introduced into the cage cannot be regarded as an important factor in their distribution, since the two openings through which they entered the cage were placed at an equal distance of 30 cm. from either end of the cage.

Although the reason for this peculiar distribution is unknown, it is obvious that it tends to be constant throughout all the tests. In Appendices II and III this is proved statistically. Appendix II shows that the variations in the proportion of mosquitoes found in the end compartment, from one test to the other, are statistically insignificant and may be considered as accidental.

Appendix III shows that although the mosquitoes had been given the opportunity of flying freely during the 10 min. which elapsed between the two counts (the one made 10 min. and the other immediately before the experiment), the numbers in the end compartment remained almost constant. This shows that if the mosquitoes are not subject to a particular stimulus, only a small percentage changes position in the cage within a short period of time.

Summing up, we may therefore conclude, that mosquitoes not subject to an external stimulus showed a definite tendency to adopt a peculiar and constant distribution in the cage, in such a way that their percentage in the experimental compartment was 14·6 in series I–VI and 7·8 in series VII and VIII.

2. *The number of mosquitoes in the end compartment after exposure to the stimulus*

If the bait had not exerted any influence on the mosquitoes, their number in the end compartment, after 15 min. of exposure to the stimulus, would have been approximately the same as before and should have been expected to approximate the number shown in line 4 of Table 1. These figures were obtained by multiplying the total number of mosquitoes in the cage in each experimental series by the 'constant probability' for a mosquito to be found in the experimental compartment, if no external stimulus had been present. The actual number of mosquitoes is seen in line 3 of Table 1. Line 5 clearly proves that this number was in

* In the experiment with the cage in a vertical position the compartment at the lower end was used for the experiments.

Table 1

Series of experiment no.	I	II	III	IV	V	VI	VII	VIII
Cage:	Horizontal						Vertical	
Position of human 'bait' in relation to cage	Facing	Back to cage	Facing	Outside the room	Facing	Back to cage	Beneath	Beneath
Expired air	Left	Left	Led outside	Led inside	Led outside	Left inside	Left inside	Led outside
Air current	None	None	None	None	Over whole body	On left arm	None	None
(1) No. tests	12	4	12	9	10	9	12	13
(2) Total number mosquitoes in the cage in all tests of the series (n)	566	195	566	431	523	420	640	765
(3) No. of mosquitoes in experimental end compartment after exposure in all tests of the series (r)	264	20	80	213	205	102	176	228
(4) Expected no. had the stimulus exerted no attraction (np) p (series I–VI) = 0·146 p (series VII–VIII) = 0·078	82·6	28·5	82·6	62·9	76·4	61·5	49·9	59·7
(5) Difference between the actual and expected no. in end compartment ($r-np$)	+181·4	−8·5	−2·6	+150·1	+128·6	+40·5	+126·1	+168·3
(6) Index of attraction $\frac{(r-np)}{(n-np)}\%$	37·5	0	0	40·8	28·8	11·3	21·4	23·9

series I and IV–VIII larger than expected, indicating that in the above series the influence of the bait was definitely active, attracting the mosquitoes to the end compartments.

However, for a proper evaluation of these figures we have to ascertain: (*a*) whether the increase in the number of mosquitoes found in the experimental compartments is significant. Appendix IV replies to this question in the affirmative; (*b*) whether this increase is of the same height in the various series.

In order to gauge the effect of the stimulus a simple statistical index (varying between 0% in case of non-attraction and 100% in the case of maximal attraction) may be adopted (the method by which this was done is given in Appendix V).

The indices for the various series are given in line 6 of Table 1.

3. *The attraction of mosquitoes by human beings*

The following conclusions as to the extent of attraction exerted on mosquitoes by human beings may be drawn from a study of the indices outlined above.

(1) *The air expired by the human being attracts mosquitoes, as evidenced by the following facts.*

(*a*) A human bait facing the cage exerts a strong attraction on the mosquitoes (cf. series I).*

(*b*) Approximately the same degree of attraction is exerted by the air expired by a person outside the room but conveyed into the room (cf. series IV).

(*c*) On the other hand, if the human bait is not facing the cage (series II) or, if facing it, the expired air is led outside (series III) no attraction on the mosquitoes is noted.

(2) *It is not only the expired air which attracts mosquitoes but also other factors emanating from the rest of the human body (though probably with lesser intensity).*

This is proved in the following way:

(*a*) An air current directed towards the cage and

* For this experimental series, the attraction exerted on the mosquitoes cannot only be gauged by an index showing the *increase* of their number in the experimental compartment, but also by means of an index showing the *decrease* of their number at the opposite end of the cage (cf. Appendix VI).

not passing over a human being exerts no influence on the mosquitoes (cf. Appendix VII).

(*b*) On the other hand, if the air current passes over a human being it exerts a considerable attraction on the mosquitoes (series V). The attraction was due in this case to factors conveyed to the cage by an air current not consisting of expired air since this had been led out of the room.

(*c*) A less pronounced influence was ascertained in series VI in which the air current did not pass over the whole human body (as in series V) but only over the left arm. It is probable that the difference between the extent of the attraction found in series V and VI is related to the size of the area of the human body exposed to the air current.

(3) *In the absence of a horizontal air movement the attraction is exerted in a vertical direction too, the attracting factor probably being carried upward with the column of warm air rising from the human body.*

This is proved by the fact that in series VII and VIII the index of attraction ascertained indicated a medium intensity. No significant difference was found between the experiments in which the expired air was led outside (series VIII) and those in which it remained inside the room (series VII).

(4) *In the above experiments hungry female* Anopheles elutus *were attracted towards a human bait at a distance varying from* 30 *to* 330 *cm. The time of exposure throughout was* 15 *min. (It is conceivable that, had the period of exposure been longer, the attraction would have been more intense.)*

The statistical method suggested in this article might, in future experiments, provide a means of measuring:

(*a*) The 'velocity of attraction' by calculating indices of the attraction exerted during the first 5, 10, 15, 20, ... min. of exposure to the stimulus.

(*b*) The relation between the distance at which the stimulus is placed and the intensity of attraction (cf. Appendix VI).

(*c*) The relation between the size of the stimulus and the intensity of attraction.

The question of the size and shape of the cage should also be thoroughly studied and the factors influencing the distribution of the mosquitoes within the cage in the absence of any external stimulus should be discovered.

The temperature factor should also be studied but does not seem to be of primary importance. In this connexion it should be noted that in the experiments in which the expired air was led into the room through a rubber tube 5 m. long it was certainly cooled down to a considerable degree, but this had no effect on the attracting force of the expired air.

The technique suggested should also provide an opportunity of measuring the effects of repulsive substances on mosquitoes.

SUMMARY

Anopheles elutus females are attracted by human beings. The human body eliminates certain substances which exert an attraction. The expired air alone is sufficient to attract the mosquitoes. Mild air currents may play an important part in carrying and directing these attracting factors towards the mosquitoes.

APPENDIX I

Table 1

Total no. of mosquitoes in the experimental end compartment

A, 10 min. before experiment; *B*, immediately before experiment; *C*, after 15 min. exposure

Ordinal no. of test in the series	Total no. of mosquitoes in the whole cage								Series no.																									
	Series no.								I			II			III			IV			V			VI			VII			VIII					
	I	II	III	IV	V	VI	VII	VIII	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
1	52	50	32	55	24	45	55	50	4	4	16	7	7	7	5	5	4	11	11	22	2	3	11	5	6	10	2	3	13	5	5	12			
2	46	45	24	22	66	45	55	50	4	4	14	5	5	3	3	3	3	4	4	14	10	11	15	6	5	11	4	4	14	5	6	14			
3	24	45	64	48	52	45	55	100	3	3	15	3	6	4	8	10	11	14	14	24	4	4	27	6	5	9	5	3	13	6	3	32			
4	50	55	48	51	63	50	55	100	9	9	23	10	6	6	14	7	6	11	12	30	14	12	24	6	7	12	3	3	17	7	7	27			
5	44	—	44	50	50	50	55	100	6	7	31	—	—	—	9	8	5	10	15	28	3	3	5	7	7	13	3	2	15	7	7	32			
6	43	—	58	50	60	50	55	50	6	6	22	—	—	—	9	12	8	7	8	23	2	3	27	9	11	16	4	3	14	4	4	11			
7	50	—	53	48	51	45	55	50	5	4	12	—	—	—	11	6	10	8	10	32	2	2	25	4	5	9	5	3	17	7	8	19			
8	64	—	51	55	50	45	55	50	11	12	20	—	—	—	8	11	15	8	7	25	11	10	27	4	4	10	4	3	15	6	6	17			
9	60	—	41	52	51	45	50	40	8	10	40	—	—	—	5	10	8	7	4	15	5	5	20	6	7	12	4	3	13	5	5	10			
10	51	—	39	—	56	—	50	40	13	10	24	—	—	—	8	4	4	—	—	—	7	7	24	—	—	—	3	2	12	4	5	12			
11	40	—	52	—	—	—	50	40	9	10	30	—	—	—	3	6	4	—	—	—	—	—	—	—	—	—	6	2	16	2	3	11			
12	42	—	60	—	—	—	50	50	6	5	17	—	—	—	6	5	2	—	—	—	—	—	—	—	—	—	5	4	17	7	5	14			
13	—	—	—	—	—	—	—	45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4	17			
Total	566	195	566	431	523	420	640	765	84	84	264	25	24	20	89	87	80	80	85	213	60	60	205	53	57	102	48	35	176	69	68	228			

APPENDIX II. *The variations from one test to the other in the proportion of mosquitoes found before the experiment in the end compartment are merely accidental*

Summing up separately the results of series I–VI (cage in horizontal position) and series VII and VIII (cage in vertical position) we find:

	No. tests	Total no. mosquitoes in the cage	Total no. and % mosquitoes in the end compartment: 10 min. before experiment	Total no. and % mosquitoes in the end compartment: Immediately before experiment
Series I–VI	56*	2701	391 = 14·5 %	397 = 14·7 %
Series VII–VIII	25	1405	117 = 8·3 %	103 = 7·3 %

* Tests nos. 10 and 11 of series VI were discarded, data for them being incomplete.

Proof has to be provided for the hypothesis that—before being exposed to a stimulus—the probability for a mosquito to be found in the end compartment is constant throughout all the tests. The best estimates of such constant probability are those derived from the statistics referred to above, i.e. for series I–VI: 0·146 (average of 0·145 and 0·147), for series VII–VIII: 0·078 (average of 0·083 and 0·073).

In order to test this hypothesis we (*a*) calculated in each test the difference between the actual number of mosquitoes found in the end compartment and the theoretical number which was to be expected in accordance with the theoretical probability, (*b*) squared the differences, and (*c*) divided the squares of the differences by the theoretical number. The ratios thus ascertained were then summed up. In the same way we calculated the sums of the ratios

$$\frac{(\text{Actual no.} - \text{theoretical no.})^2}{\text{Theoretical no.}}$$

for the number of mosquitoes found in the rest of the cage in each test. By adding these two sums of ratios together we obtained the following χ^2 (Table 2).

The χ^2 as compared to the corresponding numbers of degrees of freedom are, in general, small; the probability of accidentally finding larger χ^2 than those indicated in Table 2 is, in general, higher than 0·05 and we may, therefore, safely consider as correct our hypothesis that the probability for a mosquito to be found in the end compartment is constant. The only exceptions are series IV ('immediately before experiment') and series V ('10 min. before experiment' and 'immediately before experiment'). For these series the χ^2 are rather high. In series IV we do not know to what this may be due, but in series V we know that conditions were not exactly the same as in the other series (an air current having been directed towards the cage in the preliminary period, before the experiment (cf. Appendix VII)).

Table 2

Series	No. tests in series	Degrees of freedom	'10 min. before experiment': χ^2	'10 min. before experiment': Probability of finding larger χ^2 by chance	'Immediately before experiment': χ^2	'Immediately before experiment': Probability of finding larger χ^2 by chance	All tests together '10 min. before experiment' and 'immediately before experiment': Degrees of freedom	All tests together '10 min. before experiment' and 'immediately before experiment': χ^2	All tests together '10 min. before experiment' and 'immediately before experiment': Probability of finding larger χ^2 by chance
I	12	11	12·19	0·349	11·59	0·396	23	23·78	0·416
II	4	3	3·36	0·348	1·12	0·775	7	4·48	0·722
III	12	11	17·23	0·102	10·85	0·456	23	28·08	0·212
IV	9	8	13·20	0·106	26·33	0·0009	17	39·53	0·002
V	10	9	22·75	0·007	17·66	0·0398	19	40·41	0·003
VI	9	8	3·80	0·873	4·89	0·769	17	8·69	0·948
VII	12	11	4·30	0·958	6·15	0·862	23	10·45	0·988
VIII	13	12	9·92	0·633	13·80	0·314	25	23·72	0·536

APPENDIX III. *The number of mosquitoes in the end compartment is almost the same 10 min. before the experiment as immediately before the experiment*

This is seen by one glance at Table 1. However, the following may be added in proof of this:

(1) The percentage of mosquitoes in the end compartment showed the following differences

between the counts made 10 min. before experiment and those made immediately before experiment.

Table 3. *In the end compartment*

Series	Percentage of mosquitoes: 10 min. before experiment	Percentage of mosquitoes: Immediately before experiment	Difference
I	14·8	14·8	0·0
II	12·8	12·3	0·5
III	15·7	15·4	0·3
IV	18·6	19·7	1·1
V	11·5	11·5	0·0
VI	12·6	13·6	1·0
I–VI	14·5	14·7	0·2
VII	7·5	5·5	2·0
VIII	9·0	8·9	0·1
VII–VIII	8·3	7·3	1·0

(2) Let us now consider separately the differences in each test between the number of mosquitoes in the end compartment '10 min. before experiment' and 'immediately before experiment'. In Table 4 these differences are tabulated and it can be seen in how many instances the difference between the two successive counts in the same test was 0, 1, 2, 3, ... A slight correction of these numbers is given in the second part of the table which shows what would have been the difference between the two successive counts in the same test, if the total number of mosquitoes in the cage had been 50 in all experiments ('reduced series', Charlier's method: 50 approaches the actual arithmetic average of the number of mosquitoes in each test). *To form an idea as to whether these differences are small*, we used the following method: we pooled together the 'reduced' counts of mosquitoes in the experimental compartment which were made '10 min. before experiment' and 'immediately before experiment'. Then we calculated the frequency distributions of the tests in which 2, 3, 4, ... mosquitoes were found in the experimental end compartment (Table 5).

It can be shown that these frequency distributions are not far from normal (cf. also Appendix II). Their averages and standard deviations are as follows:

	For series I–VI	For series VII–VIII
No. mosquitoes in one test	50	50
Constant probability of finding a mosquito in end compartment (p)	0·1452	0·0800
Average no. mosquitoes in the end compartment ($np = 50p$)	7·26	4·00
Actual standard deviation	$\sqrt{8·41} = 2·90$	$\sqrt{2·04} = 1·43$
Theoretical standard deviation $\sqrt{[np\,(1-p)]}$	$\sqrt{6·21} = 2·49$	$\sqrt{3·68} = 1·92$

Table 4. *Number of tests in which the differences between number of mosquitoes '10 min. before experiment' and 'immediately before experiment' were found*

	Difference: 0	1	2	3	4	5	6	7	8	Total no. tests	Quadratic of the mean differences
Series I–VI	19	20	5	6	2	3	—	1	—	56	—
Series VII–VIII	9	11	3	1	1	—	—	—	—	25	—
After reduction to 50 of the total number of mosquitoes in each test:											
Series I–VI	20	18	8	3	3	2	1	—	1	56	2·21
Series VII–VIII	9	12	3	1	—	—	—	—	—	25	1·14

Table 5. *Number of mosquitoes in the end compartment*

	Number of tests in which the 'reduced' number of mosquitoes indicated below were found before the experiment*: 2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
No. of tests in series I–VI	3	5	14	9	18	19	12	9	9	6	6	—	—	4	114
No. of tests in series VII–VIII	5	12	15	8	7	2	1	—	—	—	—	—	—	—	50

* Irrespective of whether '10 min. before experiment' or 'immediately before the experiment'.

Let us suppose now that in the 10 min. that passed between the two counts there had been much flying about of the mosquitoes and that consequently there was no relation at all between the numbers found in the two counts. In that case we would have found that the frequency distribution of differences between the two numbers was also normal, and that the quadratic mean of these differences was about $\sqrt{(2\sigma^2)}$. Therefore, taking the actual standard deviation as a basis for calculation of the quadratic mean of the differences we would have obtained the following values:

For series I–VI $\sqrt{16·82} = 4·1$.

For series VII–VIII $\sqrt{4·08} = 2·02$.

Taking as a basis for calculation the theoretical standard deviation the quadratic means of the differences would have been:

For series I–VI $\sqrt{12{\cdot}42} = 3{\cdot}5$.

For series VII–VIII $\sqrt{7{\cdot}36} = 2{\cdot}71$.

Actually, the quadratic means of the differences, as given in the second part of Table 4, are much smaller, being 2·21 for series I–VI, and 1·14 for series VII–VIII. This suggests that the number of mosquitoes 'immediately before the experiment' tends to remain the same as it had been '10 min. before the experiment'.

APPENDIX IV. *The increase in the number of mosquitoes in the end compartment after exposure to the experimental stimulus is statistically significant*

Had the stimulus exerted no attraction at all, we would have found, in the experimental compartment after 15 min. exposure, about 14·6% of the mosquitoes (in series I–VI) and 7·8% (in series VII–VIII). On the basis of this hypothesis the χ^2 have been calculated as in Appendix II.

Table 6

Series	No. of tests in the series	No. of degrees of freedom	χ^2	Probability of finding by chance a larger χ^2
I	12	11	592·41	0·000
II	4	3	4·10	0·252
III	12	11	20·50	0·394
IV	9	8	463·90	0·000
V	10	9	325·18	0·000
VI	9	8	35·66	0·000
VII	12	11	354·53	0·000
VIII	13	12	536·90	0·000

The hypothesis that the number of mosquitoes found in the experimental compartment after 15 min. exposure to the stimulus is a chance finding is definitely disproved for series I, IV, V, VI, VII, VIII, while, on the other hand, it appears that for series II and III (in which the stimulus may be considered as ineffective) the hypothesis may hold true.

APPENDIX V. *'Index of attraction' and 'repulsion'**

Let

The total number of mosquitoes in the cage $= n$

The probability for a mosquito to be in the experimental compartment if no stimulus is exerted $= p$

The actual number of mosquitoes in the experimental compartment after exposure to the stimulus $= r$

Then

np = theoretical number of mosquitoes in the experimental compartment in the event of 'no attraction being exerted by the stimulus'.

$r-np$ = increase in the number of mosquitoes in experimental compartment due to the stimulus (not taking into consideration fluctuations due to chance).

$n-np$ = maximum possible increase in the number of mosquitoes in the experimental compartment—due to the stimulus (if all mosquitoes were to settle in the experimental compartment).

$\frac{r-np}{n-np}$ = index of attraction varying from 0% in case of non-attraction to 100% in case of maximum attraction.

A negative index indicating the influence of a repulsive substance would be the following:

$$\frac{np-r}{np},$$

which varies from 0% in the case of non-repulsion (np mosquitoes in the experimental compartment) to 100% in the case of maximum repulsion (no mosquitoes in the experimental compartment).

APPENDIX VI. *The reduction in the number of mosquitoes in the compartment opposite the experimental compartment in series I after exposure to the stimulus*

For series I the following data are available:

Table 7

No. mosquitoes in the opposite compartment	No. of test 1	2	3	4	5	6	7	8	9	10	11	12
Before the experiment	15	35	12	18	17	21	20	19	25	21	18	13
After the experiment	20	20	3	6	7	10	18	7	19	15	8	10

* The formulae of these indices agree with those suggested, 40 years ago, by Prof. Benini for measuring attraction and repulsion in the distribution of marriages by nationality, profession, etc. of husband and wife.

On the basis of these data, a more complete picture of the effect of the attraction exerted on mosquitoes by human beings in the twelve tests of series I is obtained as follows:

	No. mosquitoes		Resultant increase or decrease (%)
	Before experiment	After experiment	
Experimental compartment	84	264	+214
Intermediate compartment	248	159	− 36
Opposite compartment	234	143	− 39
Total	566	566	

From the above data it is seen that a decrease in the number of mosquitoes was also found in the end compartment opposite the experimental compartment. There was also a marked decrease in the intermediate compartment, which was even greater than would appear from the above percentages, since probably a part of the mosquitoes found in the intermediate compartment after 15 min. exposure was, prior to the experimental period, in the section opposite the experimental compartment and was attracted to the intermediate compartment by the stimulus.

APPENDIX VII. *An air current directed towards the cage and not passing over a human being does not exert any attractive influence on the mosquitoes*

In series V an air current was directed towards the cage before the onset of the experimental period. Had this air current attracted the mosquitoes to the end compartment, we should have found that their number in that part was higher than in the other series of tests. But from Table 8 it appears that, on the contrary, their number was *lower*: in fourteen out of twenty instances it was lower than the expected number calculated on the basis of the average percentage of mosquitoes found in the end compartment throughout the series I–VI. Summing up all the results of tests made in series V, only a total of 120 mosquitoes was found in the end compartment, instead of an expected number of 153. As shown in Appendix II the differences between the theoretical and actual numbers do not seem to be due to chance and seem to indicate that an air current has a repulsive rather than an attractive influence.

Table 8. *Number of mosquitoes in the end compartment (series V)*

Test no.	10 min. before experiment (*a*)	Immediately before experiment (*b*)	Expected no. (*c*)	Differences (*a*)–(*c*)	Differences (*b*)–(*c*)
1	2	3	3·5	−1·5	−0·5
2	10	11	9·6	+0·4	+1·4
3	4	4	7·6	−3·6	−3·6
4	14	12	9·2	+4·8	+2·8
5	3	3	7·3	−4·3	−4·3
6	2	3	8·8	−6·8	−5·8
7	2	2	7·4	−5·4	−5·4
8	11	10	7·3	+3·7	+2·7
9	5	5	7·4	−2·4	−2·4
10	7	7	8·2	−1·2	−1·2
Total	60	60	76·3	−16·3	−16·3

REFERENCES

REUTER, J. (1936). *Proefschr. Rijksuniv. Leiden*, 118 pp.

VAN THIEL, P. (1935). *Geneesk. Tijdschr. Ned.-Ind.* **75**, 2101.

VAN THIEL, P. (1937). *Bull. Soc. Path. exot.* **30**, 193.

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THE LARVAL STAGES OF THE NEMATODE *MERMIS NIGRESCENS*

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(With 11 Figures in the Text)

In a former paper (Baylis, 1944) an account was given of some observations on the life-history of *Mermis nigrescens*, and of its occurrence, naturally and experimentally, in earwigs (*Forficula auricularia*). As stated in that paper, the material then available did not seem to warrant an attempt to describe the larval development. Further material has now been obtained and studied, and it seems desirable to place on record some of the observations made, although many points still remain obscure.

On 13 June 1944, twelve earwig nymphs, which had been kept without food for 24 hr., were fed in the evening with eggs of *Mermis nigrescens*, spread in moderate numbers on a small piece of lettuce-leaf, not a scrap of which remained on the following morning. The earwigs were afterwards killed and dissected at intervals up to the 24th day, so that the larvae of *Mermis* recovered from them were of known age. Ten of these earwigs showed evidence of infection, and in the two in which no larvae were found it is probable that they were overlooked because of their very small size. When present, the larvae were generally numerous.

In the following notes, where an exact age is not ascribed to the worms, the reference is to earlier experiments in which the hosts had access to infective material for longer than 1 day, and consequently the age of the worms is only approximately known.

On 26 June 1945, through the kindness of Dr G. Lapage, a number of living earwig nymphs were received which had been collected from a plot of ground at Cambridge on which *Mermis* was known to occur. During the next few days ninety-seven of them were dissected, and sixteen were found to contain larvae of various sizes but, of course, of unknown age. The maximum number of larvae found in one of these naturally-infested earwigs was four. This number occurred on two occasions, the length of the worms being about 38–45 mm. in one case and about 20–30 mm. in the other. The longest worm found measured 69 mm.

Little is known at present of the development of *Mermis nigrescens* during its parasitic phase. The parasitic larvae have been studied by Linstow (1892) and by Hagmeier (1912), but their observations refer mainly to the later stages. Christie (1937), writing of the form known in America as *M. subnigrescens* (which, in the writer's opinion, is identical with *M. nigrescens*) says 'the parasitic development of the larva has not been carefully studied, but it seems essentially the same as that of *Agamermis decaudata*'. Of the development of *A. decaudata*, Christie (1936) has given a detailed account. There is, however, a very important difference between the life-histories of *A. decaudata* and *Mermis nigrescens*. The eggs of the former are laid in the soil, where they hatch, and the larvae, which have to find their way into a suitable host by their own efforts, reach a much more advanced state of development before hatching than do those of *M. nigrescens*. A moult takes place within the egg, and the preparasitic larva, on hatching, already has a well-developed 'oesophagus', with three long 'oesophageal glands' posteriorly, and the beginnings of the peculiar organs, connected with the 'oesophagus', which have been called the 'stichocytes', or collectively the 'stichosome' (Steiner, 1933). It also has a relatively large and well-developed mouth-stylet, which apparently remains unaltered throughout the parasitic phase. An 'intestine', a nerve-ring and an excretory pore are already visible. In larvae of *M. nigrescens* none of these structures, except a minute stylet, are recognizable at the time of hatching. The eggs of this species are passively swallowed by the host, in whose gut they hatch, and the only activity immediately required of the larvae is to migrate through the gut-wall into the body-cavity. The differentiation and further development of their organs take place almost entirely after this definitive position in the host has been attained.

METHODS

The young parasitic larvae of *M. nigrescens* are extremely delicate and fragile, and their tissues and organs are of extreme transparency. The writer has experienced great difficulty in devising satisfactory methods of preparing and examining them. The usual procedure has been to dissect the host in a large drop of 1 % solution of NaCl, but this medium does not seem to suit the larvae very well. They almost immediately become motionless and soon die in it, and if left in it for more than a few minutes they rapidly show signs of degeneration, and may

burst. It was therefore found necessary to preserve them as quickly as possible after removal from the host. For this purpose no reagent was found to give better results than cold 5% formalin, acetic-formalin, or lactophenol. More elaborate fixatives, such as Schaudinn's or Carnoy's fluid, did not prove satisfactory. For examination, no really good method was found. Staining with paracarmine, either with or without subsequent counter-staining with indigo-carmine, sometimes gave fairly good results, but seldom enabled much more of the anatomy to be made out than was possible in unstained preparations. This was due partly to the fact that the stained specimens had to be mounted in balsam, which has too high a refractive index for such transparent objects. If they were mounted in glycerine, the stains were liable to be washed out. Lactophenol, and even glycerine, were found to render the smaller larvae too transparent, and often it was found best to study them, unstained, in formalin or acetic-formalin. In the older larvae the great development of the 'fat-body' or 'trophosome' obscures almost every other organ, and this proved a great obstacle to the investigation either of stained or of unstained specimens. Attempts at sectioning were disappointing, and dissection was found impracticable, but in a few cases the technique of Linstow (1892), who isolated the 'oesophagus' by cutting off the anterior portion of the living larva and pressing out its contents, was adopted with partial success. The refringent globules (or the majority of them) contained in the 'trophosome' were found to be insoluble in ether, and did not show any special affinity for the stain Sudan III: facts tending to confirm the conclusion of Rauther (1906) and of Chitwood & Jacobs (1938) that in other Mermithidae these globules are not, for the most part, composed of fat, but probably of proteins.

LARVAL DEVELOPMENT

Christie (1937), writing of *Mermis subnigrescens*, says that at the time of deposition the egg contains an infective larva 'that has molted at least once'. The evidence for the occurrence of this moult is not stated, though moulting within the egg was observed by Christie (1936) in *Agamermis decaudata*.

From Christie's figures of the process of hatching in *Mermis subnigrescens*, the larva appears to measure at this time about 0·22 mm. in length.* This agrees fairly well with the writer's observations on *M. nigrescens*, larvae of which, liberated from the egg-shell by pressure on the coverglass, measured about 0·24 mm. in length and about 0·01 mm. in maximum thickness. In these larvae (Fig. 1) no differentiation can be observed in the internal structure, which appears almost uniformly granular, with slightly clearer areas at the extremities. The only conspicuous organ is the mouth-stylet, which is about 13μ long and is of the peculiar shape indicated in Figs. 1–5 (*s.*). The posterior portion of the stylet is bifid, and has a slight sigmoid curvature towards the dorsal side. The body of the larva tapers somewhat posteriorly, but the tail-end is bluntly rounded.

Larvae found in the body-cavity of the host 2 days after infection (Fig. 2) show little further development. They have even decreased a little in length, and now measure only about 0·2 mm. Their thickness is the same as on hatching (about 0·01 mm.). The cellular contents of the body-cavity show signs of becoming arranged in a longitudinal series of irregular, solid masses, but there is as yet little that can be called differentiation of organs.

The next stage observed is the 7-days-old larva (Fig. 3). This has scarcely increased at all in length since the 2nd day, but is now considerably stouter, its maximum thickness being about 0·014 mm. Its internal structure shows a considerable increase in complexity. The anterior and posterior portions of the body still contain masses of apparently undifferentiated cells, but the middle portion is occupied by a series of four somewhat oblong masses (*st.*), each containing two spherical bodies (apparently nuclei), appearing almost as clear spaces and lying one behind the other. These masses are the rudiments of the 'stichocytes'. Immediately behind them is a central column of four or five relatively large cells (*t.*) which are the first beginnings of the 'trophosome' ('fat-body' or 'intestine'). Ventrally to this column of cells, and at about its middle, lies the genital rudiment (*g.*), which is the only other organ definitely recognizable.

By the 9th day (Fig. 4) considerable growth has taken place. The larvae now measure about 0·25–0·33 mm. in length and 0·02–0·028 mm. in maximum thickness. While the organs of the anterior region of the body still remain obscure, the 'stichocytes' are now larger and very well defined. The two large nuclei in each, which have also increased in size, have the appearance of clear rounded spaces and show a tendency to overlap. The nucleus of a large cell (*N.*) is visible in close contact with the ventral surface of the anterior stichocyte. This is probably the rudiment of two large cells which later appear in this position and are connected with the sheath of the oesophageal

* Christie's statement that 'the recently hatched larva' is about 740μ long is clearly erroneous, since he refers to a figure of a larva in a much more advanced stage of development, whose length, according to the attached scale, is about 340μ. The larva shown in this figure corresponds most closely with larvae 9 days old observed by the writer (Fig. 4).

tube. The cells of the 'trophosome' have now multiplied and form a solid column three or four cells thick. The genital rudiment is larger and more conspicuous than in the previously described stage.

After the 9th day a sudden and very marked increase is to be observed in the rate of growth. A moult apparently occurs between the 9th and 11th days. In some 9-days-old larvae (Fig. 5) an extremely delicate loose sheath of cuticle (*cu.*) can be seen at the extremities. The actual shedding of this cuticle has not been observed, but the sudden burst of rapid growth strongly suggests that a moult has occurred. During the first 9 days, as has been said, very little increase takes place in the length of the larvae, though they double their thickness. Larvae 11–13 days old, however, measure about 1·2–1·5 mm. in length and 0·06–0·07 mm. in maximum thickness. They have thus multiplied their length more than four times in 2–4 days, and their thickness is about three times as great as it was at 9 days old. A similar rapid rate of growth continues from this time until the larvae reach their full size. At 18–22 days they measure up to 5·6 mm. in length; at 31–34 days, up to 25 mm.; and by the 37th day some have attained a length of 50 mm. The rate of growth, in the later stages, evidently varies considerably, as worms of very different sizes are found together in the same host, even when their age is known to be approximately the same. The average size ultimately attained by the larvae in a single host depends, no doubt, upon the food-supply available, and this in turn upon the number of worms present. As has already been shown (Cobb, Steiner & Christie (1927); Christie (1929); Baylis (1944)) the number of worms per host also determines their sex, and those which remain small are likely to be males.

The cuticle of the larvae, in spite of their increasing size, remains extremely thin until about the 34th day, when there is a sudden and very great increase in its thickness, at least in those individuals which are destined to be males, and in which by this time (and sometimes as early as the 21st day) the rudiment of the male genital pore can be detected. It is probable that this thickening of the cuticle takes place considerably later in worms destined to be females, as these remain much longer in the host. In larvae 24 days old the cuticle is not more than 2–3μ thick, as measured in optical section. In some larvae 31–34 days old it remains the same, but in others its thickness has increased to 6–12μ, especially near the extremities. In male larvae that emerged naturally from the host when 35–37 days old, the cuticle varies in thickness, in different regions of the body, from 10 to 20μ. Shortly before emergence the cuticle seems to become loosened from the underlying tissues, especially at the extremities, evidently in preparation for the moult which, according to Hagmeier (1912), takes place several weeks after emergence.

DEVELOPMENT OF ORGANS

Longitudinal fields. There is a close correspondence between the degree of development of the trophosome and that of the lateral and ventral subcuticular 'chords' or 'fields'. These longitudinal bands, each composed of several rows* of cells with conspicuous nuclei, have already reached a fairly advanced stage of development, at least in the posterior portion of the body, in larvae 11–13 days old. They are completely differentiated in larvae 19–22 days old, when they extend throughout almost the whole length of the body. As will be seen below, the trophosome develops at almost the same rate and has a similar extent, and this fact tends to support the theory that the cells of the longitudinal fields are concerned in the collection or elaboration of food-materials, taken up by osmosis from the surrounding body-fluid of the host, and ultimately to be stored in the trophosome.

Trophosome. The trophosome, whose origin in the younger larvae has already been described, appears, in larvae 11–13 days old, as a solid cylinder of numerous small cells, extending from immediately behind the posterior stichocyte almost to the posterior end of the body. In larvae about 18–19 days old it is just beginning to grow forward so as to overlap the posterior stichocyte. During the next 2 or 3 days the trophosome begins to acquire a vacuolated appearance. By the 24th day it greatly overlaps the stichocytes, and in some larvae even extends in front of the most anterior of these organs. In specimens judged to be 31 days old it extends to within a short distance of the anterior extremity, and between the 31st and 34th days the characteristic refringent globules of food-material, hitherto not seen, begin to appear in its spaces. As some larvae emerge from the host when they are only 35–37 days old, it appears that the reserve food-material which is destined to maintain the animal for the whole of its free-living existence must all be converted into this form and stored in the trophosome within a period of 3–5 days. On the assumption (which appears well founded) that feeding takes place by absorption through the cuticle, less than 3 days may be available for this process of storage, since at about the 34th day, as we have seen, an immense thickening of the cuticle suddenly occurs, and this would probably prevent further absorption.

It should be noted that there is at no time any direct connexion between the trophosome and the

* The nuclei of the lateral fields are ultimately arranged in three rows, and those of the ventral field in two rows.

so-called 'oesophagus', the structure and development of which will next be discussed; at no time does the trophosome present the appearance of an 'intestine' with a continuous lumen; and at no time is there any indication of an anus.

The oesophageal apparatus. The structure and function of the so-called 'oesophagus' of Mermithids present a problem of great difficulty, which has engaged the attention of a number of investigators. It is well known that this apparatus differs very widely from the oesophagus of a typical Nematode, but its structure is as yet by no means fully understood. Dujardin (1842), the original describer of *Mermis nigrescens*, seems to have believed that it was continuous with the 'intestine' (i.e. the trophosome). Meissner (1856) observed that its lumen ended blindly behind, but described it as having lateral pockets (Magenhöhlen) at intervals along its course, which he believed opened into the trophosome. Linstow (1892) gave an account of the apparatus in *M. nigrescens* which, although it seems to have been fanciful in some respects, was in others fairly accurate. He confirmed Meissner's observation that the lumen (which he calls the 'Chitinrohr') ends blindly, and that the organ does not lead into an intestine. He

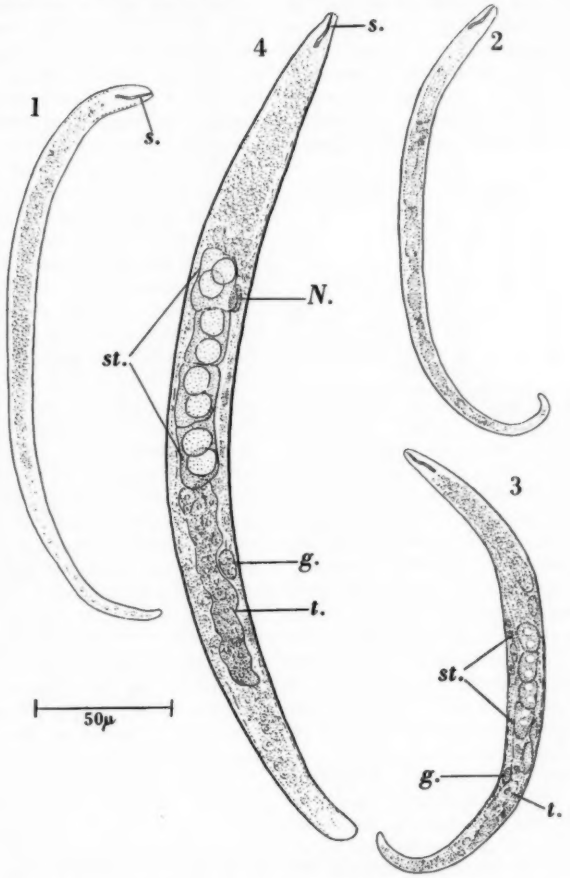

Fig. 1. *Mermis nigrescens.* Larva released from the egg-shell by pressure. *s.* stylet.
Fig. 2. Larva from body-cavity of earwig, 2 days after infection.
Fig. 3. Larva on the 7th day after infection. *g.* genital rudiment; *st.* stichocytes; *t.* trophosome.
Fig. 4. Larva on the 9th day after infection. *g.* genital rudiment; *N.* large nucleus; *s.* stylet; *st.* stichocytes; *t.* trophosome.

describes the 'Chitinrohr', however, as being enclosed in the inner core of a non-muscular sheath, which inner core has a series of spindle-shaped thickenings. Each of these, he says, contains two glandular masses, and between them a body into which the 'Chitinrohr' opens by a circular aperture.

G. W. Müller (1931) has given some account of the structure of the 'oesophagus' in various Mermithidae, but did not study it in detail in *M. nigrescens*. He describes for several other species a narrow anterior tube with a thin coat of protoplasm containing nuclei (sometimes in spindle-shaped swellings), and a posterior portion consisting

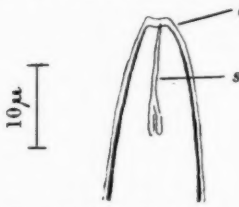

Fig. 5. Anterior end of 9th-day larva. *cu.* loose cuticle; *s.* stylet.

of a continuation of this tube and an elongate body in close association with it. This elongate body, in a form which he calls *M. locustae*, he believes to be a single cell. His diagrams indicate that the elongate body is identical with the 'stichosome' of *M. nigrescens*, in which species it is figured as containing four darker masses, presumably nuclei. Müller's conception of this organ, apparently, is that it is attached to the sheath of the oesophageal tube by its anterior end, and sometimes (though this is not indicated for *M. nigrescens*) connected at this point with a special large cell ('angelagerte Zelle'). The rest of the organ is represented in the diagrams as quite free from the oesophageal tube.

Christie (1936) describes in the parasitic larvae of *Agamermis decaudata* 16 'unicellular structures' or 'stichocytes', forming two longitudinal series of eight, between which passes the oesophageal tube. Chitwood (1935; also in Chitwood & Chitwood, 1938) claims to have demonstrated that each stichocyte in this species has an opening into the oesophageal tube, and is, in fact, an oesophageal gland. In *Mermis subnigrescens*, Christie (1937) regards each of the eight large nuclei of the stichosome as representing a separate stichocyte. As has been shown above, however, the eight nuclei in *M. nigrescens* appear from the first to be arranged in pairs, each pair being enclosed in a single protoplasmic mass. This arrangement becomes even more evident in the later stages of development, and it is possible to regard the number of stichocytes as being only four, and each stichocyte as binucleate.

The development of the stichosome, up to the 9th day, has already been described. It is, at that time, about 0·08–0·09 mm. long, and is separated by a distance about equal to its own length from the anterior extremity, and rather more than 1½ times that distance from the posterior extremity. The proportion of the body-length taken up by the oesophageal apparatus undergoes a gradual change from this time onwards, owing to the more rapid growth of the posterior region of the body. The stichosome itself increases enormously in length, but not in proportion to the body as a whole.

The first change noticeable in the stichosome is at about the 13th day (Fig. 6), when each stichocyte has begun to elongate, and its two nuclei have also assumed a slightly more elongate oval form and show an increased tendency to overlap. The nuclei now appear more densely granular than the cytoplasm of the stichocytes. By the 14th day (Fig. 7) the elongation and overlapping have become more pronounced. The two nuclei of the anterior stichocyte sometimes assume a parallel position, as shown in the figure, but usually the two nuclei of each stichocyte become flattened out and tapered at their overlapping ends so as to leave an oblique cytoplasmic partition between them. The extreme phase of this process is seen in Fig. 10. In the 14th-day larva two large nuclei (Fig. 7, *N.*, *N.'*) can be seen in close apposition to the anterior stichocyte near its two ends. They appear to be enclosed in a vaguely-defined sheath or strand of protoplasm (*sh.*), but the relationships of this are difficult to make out at this stage. At a slightly later stage, when the larvae are 19–22 days old (Fig. 8) it is possible, in favourable specimens, to trace the structure containing these two large nuclei forward to the mouth. It appears still as a vaguely-defined strand of protoplasm, difficult to see among the crowded mass of cells in the anterior region of the body, but it contains along its course five or six more, rather smaller, oval or spindle-shaped nuclei (*n.*, *n.*). At its extreme anterior end there appears to be a faint suggestion of a lumen. This apparently still contains the original larval stylet, which can be recognized even in larvae about 31 days old.

The structure just described is evidently the sheath of the future oesophageal tube. In later stages (Fig. 9) its free portion (i.e. the portion anterior to the stichosome) gradually becomes elongated, and the nuclei become distributed singly along it at more or less regular intervals, not only in the free portion (in which each nucleus forms a slight swelling) but in that which runs back in close association with the stichosome almost to its posterior extremity. The number of these nuclei is possibly constant, but has not been definitely ascertained. There are some six or seven (exclusive of the two giant cells) in the region of the stichosome, and at least seven along the course of the free

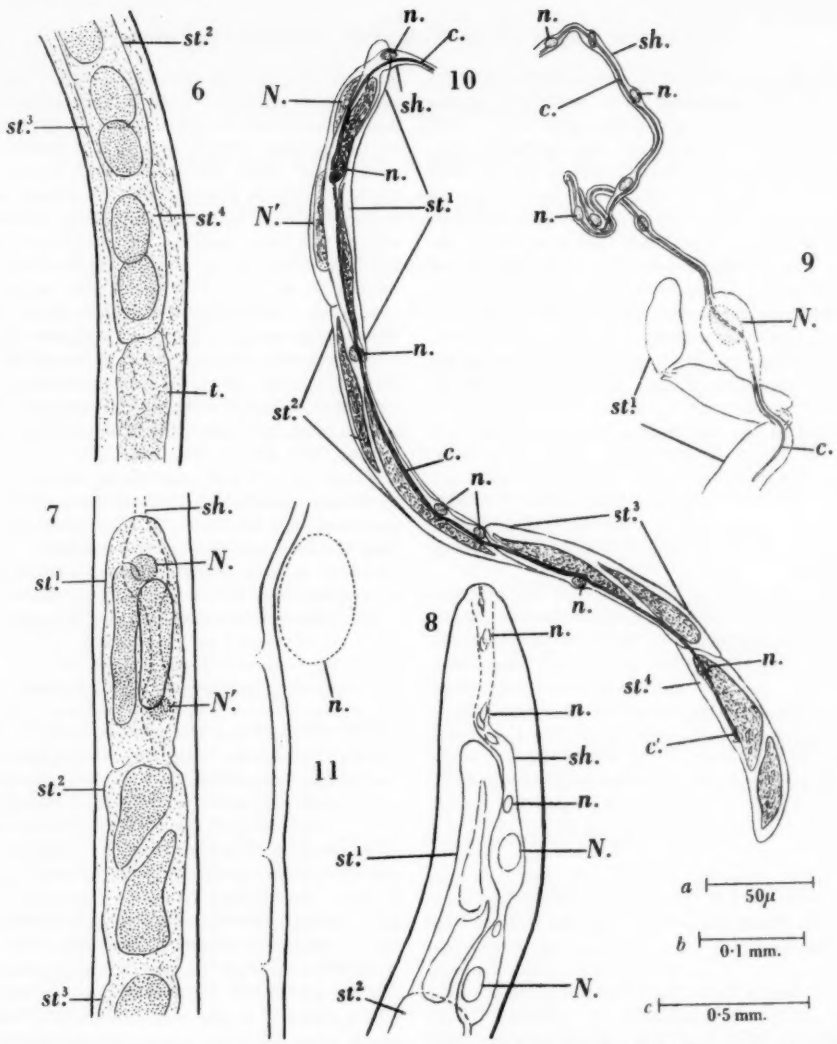

Fig. 6. Portion of 13th-day larva. *st.*³ posterior part of second stichocyte; *st.*², *st.*⁴ third and fourth stichocytes; *t.* trophosome.

Fig. 7. Portion of 14th-day larva. *N.*, *N′.* the nuclei of the two large cells of the oesophageal sheath; *sh.* oesophageal sheath; *st.*¹, *st.*² the two anterior stichocytes; *st.*³ part of third stichocyte.

Fig. 8. Anterior end of larva 19–22 days old (composite and semi-diagrammatic drawing). *N.*, *N.* the two large nuclei, and *n.*, *n.* small nuclei of the oesophageal sheath (*sh.*); *st.*¹ anterior stichocyte; *st.*² part of second stichocyte.

Fig. 9. Anterior portion of the oesophageal apparatus, pressed out from a fresh worm and mounted in formalin. (The larva, from a naturally-infested earwig, was of unknown age, and was not measured, but was almost ready to emerge.) *c.* cuticular oesophageal tube; *N.* large nucleus, and *n.*, *n.* small nuclei of oesophageal sheath (*sh.*); *st.*¹ portions of the anterior stichocyte (damaged in preparation).

Fig. 10. The complete posterior portion of the oesophageal apparatus, pressed out from a fresh worm and mounted in formalin. (The larva, from a naturally-infested earwig, was about 40 mm. long.) *c.* cuticular oesophageal tube; *c.′* its blind end; *N.*, *N′.* large nuclei, and *n.*, *n.* small nuclei of oesophageal sheath (*sh.*); *st.*¹–*st.*⁴ the four stichocytes.

Fig. 11. Portion of the cuticular oesophageal tube, in the region of the anterior stichocyte, showing indications of possible lateral branches. *n.* outline of one of the small nuclei of the sheath.

Scale *a* refers to Figs. 6, 7 and 11; scale *b* to Fig. 8; scale *c* to Figs. 9 and 10.

portion of the oesophageal sheath. Apparently the last part of the apparatus to be laid down is the narrow cuticular duct, or 'oesophageal tube'. It is difficult to say exactly when this becomes fully developed, but its anterior part at least begins to be recognizable in larvae about 31 days old. It may be that the original larval stylet is incorporated in it. There is, at least, no evidence that a moult occurs at which the old stylet might be shed, and it has been pointed out (Baylis, 1944) that up to the time of emergence from the host a minute stylet is still present just within the anterior extremity of the tube.

In male specimens just previously to emergence, when they are about 40 mm. long, the stichosome (Fig. 10) attains a length of about 3·4 mm., and the free portion of the oesophageal tube anterior to it (Fig. 9) is about 2 mm. long. Thus the whole oesophageal apparatus finally occupies roughly one-eighth of the total length of the worm. Measurements of the apparatus in the full-grown female larva are not available, and may possibly be somewhat greater.

Here and there along the course of the cuticular tube, where it runs in close contact with the stichocytes, faint suggestions of lateral openings have occasionally been detected under high magnification (Fig. 11). Possibly these may be the beginnings of minute ducts leading into the substance of the stichocytes, such as have been figured for *Agamermis decaudata* by Chitwood & Chitwood (1938).

The history of the development of this remarkable apparatus raises once more the question of its possible function. In spite of its obviously great differences from a typical Nematode oesophagus, the prevailing view still seems to be that it is concerned in some way in the process of nutrition. Thus Chitwood & Chitwood (1938), after remarking that 'the lumen does not connect with the degenerate intestine', say '. . . it is difficult to believe that fluid food is not drawn in through the oesophagus but if so, we do not know its destination'. Linstow (1892) was of the opinion that the apparatus was 'analogous but not homologous with the oesophagus of [typical] Nematodes, since it obviously functions as oesophagus and simultaneously as intestine'. This double function does not, however, appear obvious to the present writer, and it is doubtful whether even liquid food could be drawn in by such an organ, devoid as it is of any musculature capable of expanding or contracting its lumen. Müller (1931) rejects the view that the apparatus serves for the intake of food, for this reason and because it has no connexion with the 'mid-gut'. He suggests, however, that it produces a secretion which serves for the 'predigestion' of food (the coelomic fluid of the host), which is then absorbed through the cuticle. He states that, when living specimens of *Mermis chrysopidis* Müller were placed in a film of dilute Chinese ink, he observed a stream of clear secretion issuing from the mouth.

There can be little doubt, in the writer's view, that the apparatus is of a glandular nature, and that its secretion must be voided through the mouth, by way of the extremely narrow cuticular tube. This cuticular tube is not formed, and the oesophageal apparatus therefore is not fully developed, until a few days before the emergence of the larva from its host, and by this time the period of feeding at the host's expense is evidently at an end. The trophosome has already reached its full dimensions, and is becoming packed with the globular masses into which the absorbed food-materials have been converted. It seems unlikely, therefore, that the oesophageal apparatus can serve the purpose even of external digestion, as suggested by Müller, and more probable that its true function is connected with the act of emergence. It may be that its secretion has a proteolytic action which assists the worm in re-entering the alimentary canal from the body-cavity of the host, since observation has shown that emergence usually takes place through the anus or through the mouth. This suggestion receives some support from the fact that the stichosome, which remains plump and extended so long as the worm is in the host, has a 'spent' and shrivelled appearance after its emergence.

REFERENCES

Baylis, H. A. (1944). *Parasitology*, **36**, 122.

Chitwood, B. G. (1935). *J. Parasit.* **21**, 225.

Chitwood, B. G. & Chitwood, M. B. (1938). *An Introduction to Nematology*, Sect. I, Pt. II. Babylon, N.Y.

Chitwood, B. G. & Jacobs, L. (1938). *J. Wash. Acad. Sci.* **28**, 12.

Christie, J. R. (1929). *J. Exp. Zool.* **53**, 59.

Christie, J. R. (1936). *J. Agric. Res.* **52**, 161.

Christie, J. R. (1937). *J. Agric. Res.* **55**, 353.

Cobb, N. A., Steiner, G. & Christie, J. R. (1927). *Off. Rec. U.S. Dept. Agric.* **6**, 43, p. 6.

Hagmeier, A. (1912). *Zool. Jb.* (Syst.) **32**, 521.

Linstow, O. von (1892). *Arch. mikr. Anat.* **40**, 498.

Müller, G. W. (1931). *Z. Morphol. Ökol. Tiere*, **24**, 82.

Rauther, M. (1906). *Zool. Jb.* (Anat.), **23**, 1.

Steiner, G. (1933). *J. Parasit.* **19**, 249–50.

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STUDIES OF ACARI

IV. A REVIEW OF THE LUNG MITES OF SNAKES

By FRANK A. TURK, Ph.D.

(With 9 Figures in the Text)

In July 1945 I received from Col. A. E. Hammerton, pathologist to the Zoological Society of London, some interesting material among which were acari which had been taken, at different times, from the respiratory system of various snakes during the post mortems held by him. Among the mounted preparations sent to me was a new species, described in this paper, and the other material, referable to known species, has made possible a new description of one of these and has provided me with a useful basis from which to undertake a review of the whole group, which, for purposes of comparison, has had to include the consideration of the Mesostigmatic ectoparasitic acari of Ophidia.

HISTORY OF THE GROUP

Dr F. da Fonseca (1934, 1940) has dealt briefly with the literature of the group up to the time of publication of the last of these, although two species of Entonyssidae—*Ophiopneumicola hammertoni* (Radford) and *O. bedfordi* (Radford)—were overlooked and another species—*Entonyssus vitzhumi* Schmidt—was added in the same year as the last of Dr Fonseca's papers was published.

The Mesostigmatic acari, described up to the present time as infesting snakes, may be listed very briefly: a longer account of the external parasites will be found in the first of Fonseca's papers mentioned above.

LIPONYSSIDAE

Ophionyssus natricis (Gervais, 1844), genotype

Synonyms: *Dermanyssus natricis* Gervais, 1844; *Ophionyssus natricis* Megnin, 1884; *O. natricis* Canestrini, 1891; *O. natricis*, Berlese, August 1892; *Ichoronyssus natricis* Hirst, 1915; *Liponyssus natricis* Berlese, 1918; *L. natricis* Hirst, 1921.

Ophionyssus serpentium (Hirst, 1915)

Synonyms: *Ichoronyssus serpentium* n.sp.? Hirst, 1915; *Liponyssus serpentium* Hirst, 1921; *Serpenticola serpentium* Ewing, 1922.

Liponyssus triangulus Ewing, 1922

IXODORYNCHIDAE Fonseca, 1934 = *Ixodorynchinae* Ewing, 1922

Ixodorynchus liponyssoides Ewing, 1922
Ixobiodes butantanensis Fonseca, 1934

ENTONYSSIDAE Fonseca, 1934 = *Entonyssinae* Ewing, 1922

Entonyssus halli Ewing, 1922, genotype
E. rileyi Ewing, 1924
E. glasmacheri Vitzhum, 1935
E. ewingi Hubbard, 1939
E. vitzhumi Schmidt, 1940
E. heterodontos Keegan, 1943
Ophiopneumicola colubri Hubbard, 1938, genotype
O. bedfordi (Radford, 1937), hereinafter made the type of a new genus

Synonym: *Entonyssus bedfordi* Radford, 1937.

Ophiopneumicola hammertoni (Radford, 1939)

Synonym: *Entonyssus hammertoni* Radford, 1939.

Ophiopneumicola elaphes Keegan, 1943
O. natricis Keegan, 1943

PNEUMOPHIONYSSIDAE Fonseca, 1940

Pneumophionyssus aristoterisi Fonseca, 1940

[Lists of the hosts are given in the keys on p. 22.]

Of the above families the Liponyssidae and the Ixodorynchidae include the ectoparasites of snakes whilst the Entonyssidae and Pneumophionyssidae include all the Mesostigmatic acari known to infest the respiratory system of snakes. With the exception of *Ophiopneumicola bedfordi* (Radford) all of the latter have been taken on New World species of snakes, the last named infests the Green Mamba, *Dendraspis angusticeps* Smith, from Africa.

RELATIONSHIPS OF THE FAMILIES AND GENERA

When Ewing (1922) described the first known lung mite of a snake and also brought forward the first *Ixodorynchus* he treated these as representative of two new subfamilies of the family Dermanyssidae. The raising of these groups to family rank by Fonseca in 1934 and 1940 is probably justified in

that the morphological differences between them and their nearest allies are as great, at least, as those which separate the Liponyssidae from the Dermanyssidae.

Clearly, all the mites with which we are at present concerned, whether ecto- or endoparasites, are very much more nearly related to the Liponyssid complex than to the Dermanyssid one. The chelae are shear-like rather than needle-like except in those genera where one of the digits has been partially or entirely lost; the genito-ventral shield is narrow and generally only slightly chitinized; the legs and their armature, the form of the palpi and the general configuration of the hypostome are on the whole more characteristic of the Liponyssidae than of the Dermanyssidae. It would seem reasonable to expect that the lung mites as a group should have descended from the mites living on the integument of snakes and it is perhaps of some significance that the species of *Liponyssus* recorded in the list above should have been taken from the body of a King Snake *Lampropeltis calligaster* (Say).

Several species of the genus *Liponyssus*, e.g. *L. biscutatus* Hirst have two dorsal shields and, as a rule, each of these bears on its posterior margin a pair of longer and occasionally stronger setae. Such an arrangement is seen in the genus *Ophionyssus*, where the posterior shield is very small and has almost completely aborted; and in various forms of the Ixodorynchidae. In *Ixodorynchus* the two shields have fused leaving only the suture whilst in *Ixobiodes* the two are quite distinct and in the deutonymph the posterior one carries on its hinder border two typical long setae. It seems almost certain, judging by the condition of these shields in the protonymph of the last-named genus and by the condition of the shields in related genera that this posterior shield is compounded by the fusion of several smaller primitive shields. It is important for the present purpose to note that the anterior shield is almost always markedly truncate or has a decided tendency to be so (cf. the state of this shield in protonymphs of *Liponyssus*).

In *Pneumophionyssus* there is one dorsal shield which covers only a very small part of the dorsum and to which, perhaps as a secondary adaptation, the fusion of a pair of the small intermediate shields has given a blunt posterior point. At the hinder end of the dorsum, but *not* on the margin and hence not to be interpreted as the longer marginal hairs found in many species of Mesostigmata, there are two longer and stronger setae which I interpret as marking the position of the aborted posterior shield. In spite of its very characteristic chela this genus stands nearer to the genera of ectoparasitic snake mites than do the other lung mites, for in this genus the stigmata are ventral, as in all the species found on the integument, and not more or less lateral, as in the Entonyssidae.

In two species of the last family the dorsal shield is large, rounded posteriorly and covers most of the dorsum and bears on its posterior border a pair of long strong setae: this occurs in *Entonyssus vitzhumi* Schmidt and *E. glasmacheri* Vitzhum. I suggest, therefore, that these two species show clearly a dorsal shield which has originated in the fusion of the anterior and posterior shields found in the ectoparasitic forms. With one exception, all the species of *Entonyssus* and *Ophiopneumicola* have similar dorsal shields although the long posterior setae are not always apparent (the two species *Entonyssus halli* Ewing and *E. rileyi* Ewing have to be excepted from these statements as they are far too briefly described and have never been figured). By homology it can be postulated that here, too, the dorsal shield was formed by the fusion of the two primitive shields.

The single exception mentioned above is *E. bedfordi* Radford, which has been made the type of the new genus *Hammertonia*. This species has a single dorsal shield covering only the anterior part of the body, sharply truncate behind and bearing on its posterior border two long strong setae. According to the view set out above, this structure must represent the single anterior shield, not changed from its original shape and structure. This at once distinguishes the species from all the other lung mites of snakes described hitherto.

Thus the Entonyssidae are probably derived from ancestral forms not very different from those found as ectoparasites of snakes at the present time. These originally had the two or possibly more dorsal shields which are characteristic of the more primitive Liponyssid species. Life in the respiratory system of snakes probably induced, as part of the general tendency in endoparasitic acari to lose strongly chitinized plates, three separate lines of development. In the Pneumophyionyssidae (perhaps the most primitive) the large posterior shield aborted and the anterior shield fused with the small intermediate ones. In *Ophiopneumicola bedfordi*, and perhaps in all the lung mites of African snakes, the posterior shields all aborted leaving only the anterior one in its more or less primitive condition. The rest of the Entonyssidae had all the dorsal shields fuse together with a later reduction in the size of the shield from that which is found in *Entonyssus glasmacheri* and *E. vitzhumi*.

LIFE-HISTORY AND GENERAL BIOLOGY

Very little is as yet known of this subject although a certain amount may be deduced from the facts already recorded by various authors.

It is almost certain that all the forms are vivi-

parous. Six-legged larvae have been found in *E. ewingi*, *E. glasmacheri*, and *Ophiopneumicola colubri* whilst Radford says of *O. bedfordi*: 'On one of my slides there is an adult female inside which is plainly visible an eight-legged nymph, which proves this species [genus] to be viviparous.' If this observation is correct it is an example of a phenomenon that is of extremely rare occurrence in Mesostigmatic acari. Although the birth of a protonymph stage has very occasionally been reported for *Liponyssus* forms, it is only known to occur with certainty in a bat parasite as reported by Vitzhum (1931). The palpi of the intra-uterine larvae of Entonyssidae are very large and conspicuous and may have been mistaken by Radford for legs: in any case a re-examination of this material would be useful.

This case is of added importance because it is the general opinion of acarologists that in the Pneumonyssidae (the lung mites of monkeys and of the dog) the larva moults directly into the adult stage and any nymph stage is omitted. This would appear to be the case also in the Entonyssidae (with the exception of *bedfordi*) for no one has ever seen a nymph of any of the species so far recorded. No female has ever been found with more than one larva or, in the cases where the larva had not yet developed, more than one developing egg.

Males are much rarer than the females although they have been described in the following species: *Entonyssus vitzhumi*, *E. glasmacheri*, *E. heterodontos* and *Ophiopneumicola hammertoni*, *O. elaphes* and *O. natricis*. I have not seen the males of *O. hammertoni* but from Radford's figure (1939) there is one very remarkable difference between this genus and the males so far described in the genus *Entonyssus*, this is that he shows the anal opening to be in a separate anal plate whilst in the latter genus all the ventral plates are fused together. If this character should prove to be common to the males of *Ophiopneumicola*, it will be a better and more decisive generic character than any brought forward hitherto. In Keegan's (1943) two species it proved impossible for him to make out the outlines of the ventral plate.

The chelae of all males so far known are modified to act as sperm carriers (Vitzhum calls the accessory structure a 'Spermatophorenträger') and fertilization must be in the usual Gamasoid manner, namely the introduction of the spermatophores issuing from the male genital aperture into the female aperture by means of the chelae. As is the general rule with acari, the eggs are remarkably large; Vitzhum (1935) records that a female of *Entonyssus glasmacheri* measuring 870 μ long contained an egg $462 \times 305\,\mu$. When the larva hatches from the egg there are no chitinous plates to be seen and, according to Vitzhum (1931), there are not even any slightly more chitinized granules such as are found on the larvae of *Pneumonyssus* spp.

Neither the males, eggs or larvae of the Pneumophionyssidae are known, only the female having been so far described.

Nothing is known as to how these species disseminate themselves. They do not seem to occur in very great numbers in their host: Hubbard (1939) records eleven specimens of *Entonyssus ewingi* in a single host and the same author (1938) found twenty examples of *Ophiopneumicola colubri* in one host; the largest number appears to have been the thirty-two specimens of *Entonyssus vitzhumi* found by Schmidt (1940) in a single Bull Snake. Of the sole species of *Pneumophionyssus* only two females were found. The incidence of infestation is also not high: Hubbard (1938) says that a second specimen of the host species of *Ophiopneumicola colubri*, taken at the same time and place as the one in which his specimens were found, harboured no mites. Keegan (1943) has given a long, careful and detailed list of the species of snakes in whose lungs he has sought these acari and very few of them proved to be infested. Probably too, geographical distribution will be found to play a large part in determining the incidence of infestation for, whilst *O. natricis* was found in the one example of *Natrix sipedon sipedon* (L.) from Kansas which was examined, no specimens of the mite occured in fourteen examples of the same snake taken at Iowa. Considering all these facts, and also that only one young seems to be produced at a time, the hazards of their dispersal cannot be very great. Most probably transference from one host to another takes place either during mating or during hibernation, when this occurs in the life of the snake host. It is of interest too to note that snakes, such as those of the genus *Pituophis*, which have a well-developed epiglottis are as equally well parasitized as those which are without this structure.

EFFECT OF THE PARASITES ON THE HOST

It has long been conjectured that the parasitic acari of the Ophidia may be the vectors of disease. Sambon (1913) says: 'The Ophidian tick parasites like those of mammals, birds, lizards and tortoises appear to be in many cases the means of transmission of protozoal infections from snake to snake.' I wrote to Col. A. E. Hammerton on this matter and in the course of his reply (*in litt.* dated 28 August 1945) he says: 'It is very unusual for these mites to be associated with lesions that can be caused by the parasites but of course they are not infrequently found in the air sacs or respiratory passages of snakes, birds, etc. that have died from pulmonary diseases.'

In the related group of acari, the Pneumonyssidae, which occur in the lungs of monkeys and dogs, the mites have occasionally been reported as occurring in little vesicles in the lung tissue, of which they were presumed to be the cause. Griffiths as quoted in Newstead (1906) reports the presence of acid-fast bacilli in the lungs of monkeys infested with these mites but says that they were not the tubercle bacillus. With this in mind I wrote to Col. Hammerton asking him whether there was any evidence that these lung mites of snakes were associated with reptilian tuberculosis. In his reply in the same letter from which I have quoted above he says: 'Reptilian tuberculosis may be associated with the presence of acari—as may any other pulmonary disease such as pneumonia, mycosis, etc. but I know of no experimental evidence to support the fact that any pulmonary disease is caused or carried by acari.' It is doubtful if the mere presence of the acari in the lung tissue of the snake has any harmful effect upon the host. Hubbard (1938) says that the snake which he ultimately found to be infested with *Ophiopneumicola colubri* was kept alive in captivity from 6 May 1937 until 9 October 1937 and it is the common experience in menageries that snakes which prove on post-mortem examination to be infested with these mites have lived for many months and even years, without showing any obvious ill effect.

GENERAL HOST-PARASITE RELATIONSHIPS

All mites of the families Ixodorynchidae, Pneumophionyssidae and Entonyssidae have so far proved to be specific to each host species; each time a new species of snake is found to be parasitized the parasite proves to be a new species of mite. This naturally raises the problem as to whether mites occurring in nearly related hosts are themselves nearly related. It should be mentioned, however, that these mites can exist as stray vagrants in snakes other than the specific host when snakes are kept in captivity; in this connexion see Keegan (1943). It has already been made clear that *Ophiopneumicola bedfordi* which was taken in an African snake, the Green Mamba, must be removed to a separate genus as it belongs to a different line of descent from those which are found in the snakes of the Americas.

Of the other known lung mites of snakes of the family Entonyssidae, *Entonyssus rileyi* and *E. ewingi* have both been found on species of Rattlesnake of the genus *Crotalus*. A comparison between them, however, is handicapped by the fact that *Entonyssus rileyi* was only very briefly described; the characters given by Ewing (1924) are not sufficient for the purpose, and from the condition of the types as reported by Keegan (1943) it seems likely that but little more will be added to the already published description of this species. However, the two forms seem to be almost certainly more nearly related to each other than they are to other members of the genus. Indeed, Hubbard himself (1939) says that his species may prove to be identical with *E. rileyi* and that much of his description of *E. ewingi* would apply to the latter species; but he further points out that *ewingi* differs from *rileyi* in that (1) its chelae are more complex and the movable digit is not stouter than the fixed one, (2) the abdomen is not naked, and (3) it has a larger ratio of tarsus I to tibia I. I consider that points (2) and (3) are certainly good specific differences and that both are valid species. However this may be it would seem that the mites found in the lungs of rattlesnakes are more nearly related to each other than they are to those of Colubrine snakes. *E. ewingi* and *E. rileyi* are the only species so far described that are more than a 1000μ long and they are a third larger than any other of the known lung mites.

The new species described in the present paper is important not only for some of the interesting details of its structure but also because it was taken from a snake of the genus *Elaphe*, a genus from which at least one other species of *Ophiopneumicola*, namely *O. elaphes* Keegan, has been taken. In addition to this the host of *Entonyssus glasmacheri* which was recorded in Vitzhum's original paper as *Coluber quadrivittatus* Holbrook has now been placed, by Stejneger & Barbour (1923), in the genus *Elaphe*. In this case therefore there is a larger amount of descriptive material available for comparative purposes and it is possible to compare the former species and the present *Ophiopneumicola americana mihi* with the other known species of *Ophiopneumicola*. The comparative data are given in Table 1, but no very clear-cut division emerges, for the new species seems to differ as much from *O. elaphes* as it does from the other members of the genus which are parasites of other genera of Colubrine snakes. It is nevertheless, more closely related to *Entonyssus glasmacheri* (that is if the generic character of the chelae be excepted) and it resembles it in all of the following points: (1) the palpus is very similar and bears similar appendages; (2) the dorsal shields of both species are very much alike and bear the similar pair of longer setae posteriorly; (3) the sternal shield is similar, in fact almost identical in both and is well demarcated from the condition found in all other species of *Ophiopneumicola* by having no lateral projections to the sternal shield; (4) the genito-ventral shield is flask-shaped in both species and in each it bears a single pair of well-marked setae. In all these respects, and in other minor ones, whose significance

is difficult to evaluate, they agree closely. The conclusions to be drawn from this support the view that *Coluber quadrivittatus* is to be placed in the genus *Elaphe*. In so far as the form of the sternal and genito-ventral shields is of specific importance it appears that *Ophiopneumicola americanus* is more closely related to *Entonyssus glasmacheri* than it is to *Ophiopneumicola elaphes*; and, if this is so, it may be that the hosts of both are more nearly related to each other than *Elaphe guttatus* (L.) is to *E. obsoleta* (Say).

Should this view prove to be correct then it would follow that *Ophiopneumicola* could not be retained as a good genus. In this connexion it is worth recalling that Keegan (1943) has shown that 'legs all similar' is no longer a character by which *Entonyssus* may be differentiated from *Ophiopneumicola* and a few species of both genera approach each other in the formation of the fixed digit of the chelae. It is true moreover that the peculiar 'eye spot' sensory organs on the pulvillus of the first pair of tarsi are not confined to the genus *Ophiopneumicola* (even these do not occur in every species as will be seen from Table 1) and I am of the opinion that, as the new species *Entonyssus heterodontos* Keegan, 1943 is found to possess these organs, the former genus should no longer be recognized whilst the diagnosis depends on the one fixed but this may not be found to be so in other members of the genus.

In the male there is present a separate anal shield, relatively broad to the size of the anal opening. The apical segment of the chelicerae of the male, i.e. the one bearing the digits, is somewhat swollen in the type-species and is obviously broader than the penultimate one. Type-species: *Entonyssus bedfordi* Radford, 1937 (Host: *Dendraspis angusticeps* Smith).

I at first intended to call this genus *Radfordia* in recognition of Radford's work in first diagnosing the species. The name is, however, preoccupied by *Radfordia* Ewing, 1937 for certain species of mites of the old genus *Myobia*. It seems therefore very fitting that the name of this genus should mark the debt which both Radford and myself owe to Col. A. E. Hammerton who has from time to time provided each of us with valuable acarine material.

Keegan (1943) has given keys to the species of the Entonyssidae but I had already constructed keys for the females of the known species before I was aware of his paper. My own keys depend on different characters from those chosen by him and in giving them here I hope that they will both make the present paper more complete and serve as a useful check when using the others for identification. Only the females are referred to in the key as the males

Table 1

	O. americana	*O. elaphes*	*O. colubri*	*O. hammertoni*	*O. natrix*
Fixed chela with accessory tooth	*	*	*	*	0
Last segment of palp smaller than penultimate	0	*	0	?	*
Sternal shield with lateral projections	0	*	*	*	*
Endopodal plates	*	*	*	0	*
'Eye spots' on tarsus I	*	*	0	*	*

(*Note.* * denotes that the character is present, 0 that it is absent.)

rather variable character alone. Fonseca (1940), however, is of the opinion that it should be retained.

A comparison of the species of *Ophiopneumicola* is set out in Table 1.

Genus **Hammertonia** n.g.

Tarsus I with two claws but without pulvillus. The dorsal shield sharply truncate with the posterior angles almost rectangles, it extends only a very little distance behind the 4th pair of coxae and on its posterior border it bears two longer and stronger hairs in the lateral third of the total width. The chelae of the female are extremely simple and the apex of both digits is very slightly bifid. In the type-species the movable arm is wider than the are of comparatively rare occurrence and indeed there are several species for which they are not yet described. I have retained the names of the genera *Entonyssus* and *Ophiopneumicola* although I am doubtful whether the latter should be retained as a valid genus, for the reasons which I have set out above: I think that it should not have a higher rank than that of a subgenus.

DESCRIPTION OF A NEW SPECIES OF OPHIDIAN LUNG MITE

Ophiopneumicola americana n.sp.

The female only is known. Colour white with very light brown shields which stain well with warm

picric acid. Length from the base of the capitulum to the posterior border 450 μ; greatest width 220 μ; it is thus the smallest species of the genus so far described. The dorsal shield covers a large part of the dorsum as will be seen from Fig. 1. It is covered with well-marked, elongate, transverse reticulations which are closer together towards the posterior border: this is a character which does not seem to have been recorded for any of the other species of the genus and is very apparent on these specimens which are stained with picric acid. In addition to this patterning on the dorsal shield there are a great number of minute, more highly chitinized granules which can be made out under a good $\frac{1}{6}$ in. apochromatic objective with critical illumination. They give the surface of the dorsal shield the appearance of being minutely punctate. The spines of the dorsal shield are very short and somewhat

A KEY TO THE LUNG MITES OF OPHIDIA

1. Chelae with movable arm falciform and fixed arm harpoon-like with a retrograde tooth or with both arms falciform generally without teeth or setae. Fam. Entonyssidae
–. Chelae of female with a long movable arm which is but weakly chitinized and strongly recurved outwards with neither teeth nor setae; the fixed arm aborted. Fam. Pneumophionyssidae
(The latter family has only one genus and species *Pneumophionyssus aristoterisi* Fonseca. Host: *Erythrolamprus aesculapii* (L.).)

ENTONYSSIDAE

1. Tarsus I without pulvillus; chelae of female bifid at apex; Dorsal shield broadly truncate, small extending only to a little behind coxa IV. *Hammertonia* nov. gen.
–. Tarsus I with pulvillus; chelae of female otherwise; dorsal shield rather broadly rounded or pointed and covering greater part of dorsum. 2
2. Chelae with the fixed arm harpoon-shaped with a recurved distal hook, the movable arm generally scimitar-shaped and with a 'fringed' structure at its base in some species. *Entonyssus*
–. Fixed arm of chelae not harpoon-like, movable arm as a rule straighter and never with a basal 'fringe'. *Ophiopneumicola*

Genus **Entonyssus**

1. Pulvillus of tarsus I with chitinized 'eye spot' sense ring. *Entonyssus heterodontos*
(Host: *Heterodon contortrix contortrix* (L.), Keegan)
–. Pulvillus of tarsus I without this structure. 2
2. Broadest at Opisthosoma. 3
–. Broadest at proposoma or at middle of body. 4
3. Fixed digit of chela nearly equal to the length of the shaft; 1240 μ total length. *E. ewingi* Hubbard
(Host: *Crotalus atrox* Baird & Girard)
–. Fixed digit of chela less than half the length of the shaft: 870 μ total length. *E. glasmacheri* Vitzhum
(Host: *Elaphe quadrivittata quadrivittata* (Holbrook))
4. Palpi apparently 4-segmented in female. *E. vitzhumi* Schmidt
(Host: *Pituophis sayi sayi* (Schlegel))
–. Palpi obviously 5-segmented in female. 5
5. Chelae stouter than palpi; length 1160 μ. *E. rileyi* Ewing
(Host: *Crotalus* sp.)
–. Chelae not so stout as the palpi; length less than 1000 μ. *E. halli* Ewing
(Host: *Pituophis melanoleucus* (Daudin))

Genus **Ophiopneumicola**

1. Pulvillus of tarsus I lacking the 'eye spot' sensory structure: legs I shorter than II.
Ophiopneumicola colubri Hubbard
(Host: *Coluber flagellum flavigularis* (Hallowell))
–. Pulvillus of tarsus I with a chitinized 'eye spot' sensory structure: legs I and II subequal. 2
2. Sternal shield without lateral projections. *O. americana* n.sp. Turk
(Host: *Elaphe guttata* (Linn.))
–. Sternal shield with lateral projections. 3
3. No endopodal plates. *O. hammertoni* Radford
(Host: *Thamnophis sirtalis sirtalis* (Linn.))
–. Endopodal plates present. 4
4. Claw of tarsus I equal in size to that of tarsus II. Fixed digit of chelae with a single tooth on the inner edge. *O. elaphes* Keegan
(Host: *Elaphe obsoleta obsoleta* (Say))
–. Claw of tarsus I at least twice the size of those of tarsus II. Fixed digit of chelae without an inner tooth. *O. natricis* Keegan
(Host: *Natrix sipedon piciventris* Cope)

sparse but are more nearly symmetrical in arrangement than is usual in the species of this genus. On the posterior border there are two much longer and stronger setae the significance of which has been discussed in an earlier part of the present paper; they are easily seen in the type preparation.

The shields of the ventral surface are very unusual and at first I took this form to be a nymph (tritonymph?), a view which appeared to be supported by the fact that neither of the type-specimens contained eggs and by the fact that the sternal shield had only two pairs of hairs. In view of the marked chitinization of all the scuta, however, I feel justified in regarding these forms as adults but the possibility of their immaturity must be borne in mind. A small tritosternum is present. Fig. 2 shows the highly characteristic form of the sternal shield which is without the reticulations of the dorsal shield but which has the 'punctation' effect described above. It differs from all the other known species in having only two pairs of setae; the third pair are present but lie off the shield on the unchitinized skin between the sternal and genito-ventral shields. This means that the two little scutulae which are represented by the setae have not coalesced with the rest of the sternum. The genito-ventral shield is rather large and of characteristic shape and bears one pair of small but well-marked setae: this too, has the 'punctation' marking and bears also the same kind of reticulation pattern which is found on the dorsal scutum but which is here confined to the sides and the posterior part of the shield. Near the fourth pair of legs, a little anterior to their coxae, is to be found a pair of somewhat large and well-marked endopodal plates which are similarly marked to the sternum. Near the posterior border of the second pair of legs there is a pair of very small, but moderately well chitinized, plates which are evidently also part of the endopodal series. Near the proximal border of the third pair of coxae there are two ill-defined very slightly chitinized areas, the position of which is shown in Fig. 2. These may also be a pair of endopodal plates but unless specimens are found in which they are more clearly marked their true nature must remain in doubt. In view of the separate series of endopodal plates just described, and taking into account their position, it is probable that the lateral projections of the sternum found in other species of this genus are endopodal plates which have fused with the sternal shield. The anal scutum is comparatively large and encloses a large anal opening; it has the same 'punctation' marks as described on the other shields. Of the two examples which I possess of the type, only one shows the anal scutum and there are no anal setae on it.

The chelae (Figs. 3 and 4) are more complicated than the type usually found in *Ophiopneumicola* and their form is best made out from the text-figures. The movable digit is much stouter than the fixed one and, as will be seen from Fig. 3, the apex is turned outwards and dorsally. The fixed chela has the tip turned at right angles to the shaft and a third of the distance from the apex bears another accessory tooth. Another elongate tooth, very difficult to discern, appears to lie laterally. The movable digit has a very faint fimbriate structure at the point of its articulation and above this articulation, on the shaft, there is a sharp pointed wedge-shaped piece almost like a secondary tooth. The last three described characteristics are all to be found in various species of *Entonyssus* and this is perhaps another valid reason for dropping the generic status of *Ophiopneumicola*.

The palp (Fig. 6) is four-segmented and the apical segment terminates obliquely. This segment bears a peculiar arrangement of sensory structures. In the lower half of the segment there is a long fairly thick flexible rod and just above this there is a broader two-pronged sense rod the base of which is set on the lower part of the terminal obliquity. It is perhaps significant in the light of what has been said of the two species above that this arrangement of sensory rods resembles that of *Entonyssus glasmacheri*. There are several long outstanding setae at the apex of the palp and, as in other species, there is one longer one on the inner side. There are also three peculiar chitinous ridges at the extremity arranged as shown in Fig. 6 and perhaps these also may be sensory structures. It is possible that they occur in other species but have not been described because they are more difficult to detect in them, and I have not been able to make them out in any of my other material.

The tarsus of the first pair of legs has the peculiar eye spot, or ocellar-like structures, on the pulvillus which have been mentioned before in this paper as occurring in other species. I have not been able to study this in detail in the present species as the state of the material would not allow of it but it is no doubt the same as that which I describe below for *Ophiopneumicola hammertoni*. The claws of tarsus I are strongly recurved, a character which is very marked in this species.

Legs I and II are about equal and both of these are subequal to III; legs IV are the longest. The proximal segments of the legs are armed with spines and the distal ones with setae as shown in Fig. 1. Tarsus I is $1\frac{1}{2}$ times the length of tibia I; tarsus II twice that of tibia II; tarsus III twice that of tibia III and tarsus IV $2\frac{1}{4}$ times the length of tibia IV.

The present species differs from *O. elaphes* Keegan in that the sternal and genito-ventral plates have a different conformation. The chelae

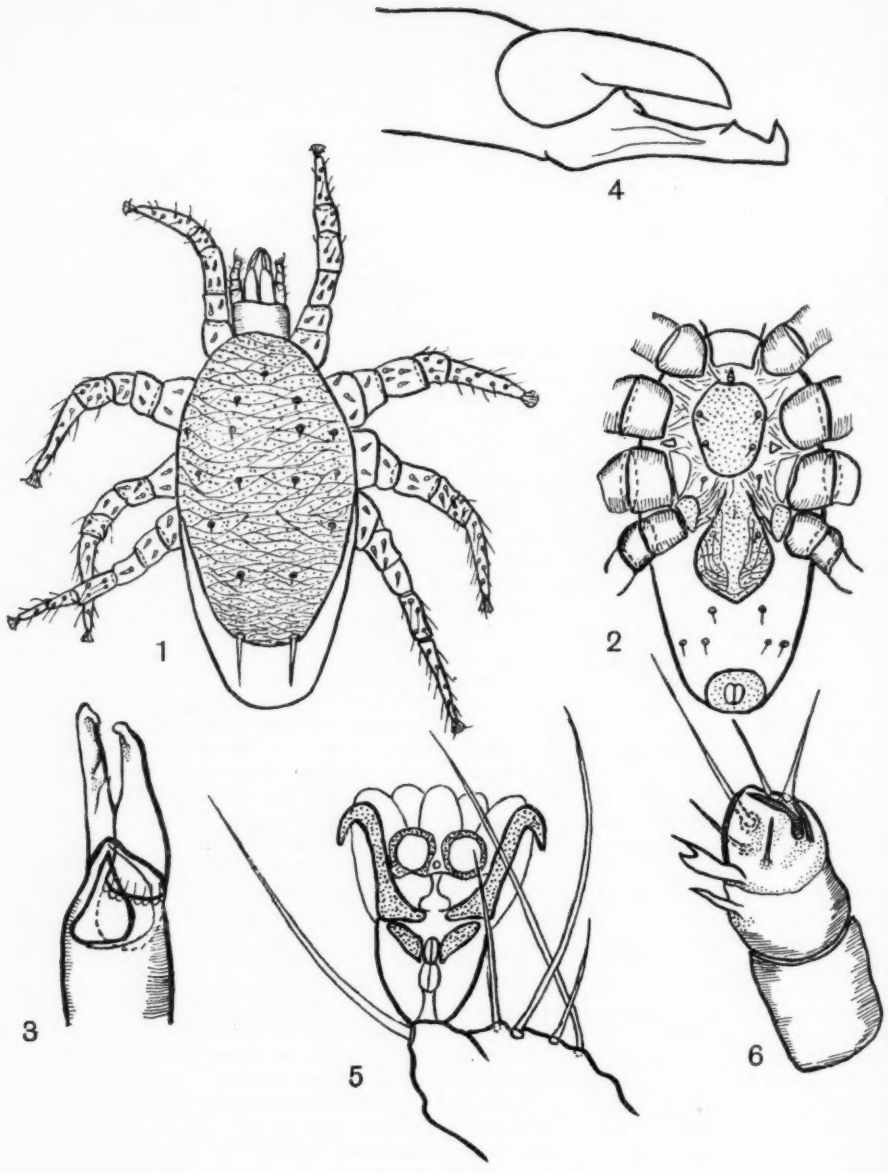

Fig. 1. *Ophiopneumicola americana* n.sp. dorsal.
Fig. 2. *O. americana* female, ventral.
Fig. 3. *O. americana* female, right chela, dorsal view.
Fig. 4. *O. americana* female, left chela, lateral from a second individual.
Fig. 5. *O. americana* female, left tarsus of first pair of legs.
Fig. 6. *O. americana* female, right palp, the two distal segments.

are more complicated in their structure in the present species and the dorsal shield carries two long posterior spines.

Type material: two females on a single slide in my own collection. Host: the Corn Snake, *Elaphe guttata* (L.)

RE-EXAMINATION AND ADDITIONAL DESCRIPTION OF *O. HAMMERTONI* RADFORD, 1939

Amongst the material sent to me by Col. A. E. Hammerton were two nicely preserved specimens of this species and since Radford's original description is very short and one or two points in it require amplification and correction I think it best to publish, in brief, the results of my re-examination.

My specimens came from the same host species as Radford's, namely the North American Garter Snake, *Thamnophis sirtalis sirtalis* (L.) and agree very well with his description in size and the shape of the genital, genito-ventral and anal shields. The dorsal shield is very similar to that of *Ophiopneumicola colubri* Hubbard but is much more pointed and leaves a good deal more of the dorsum uncovered.

Tarsus I is very much as figured by Radford but the basal part, which supports the claws and pulvillus, has a slightly different form which I have shown in Fig. 7. On comparing this with the figure given in the original description it will be noticed that the shaft of the tarsus in my specimens is more slender in relation to the pulvillus and has a peculiar prominence—better figured than described—and itself ending distally in twin knobs the relation of each to the other being best seen in Fig. 8. These cause the tarsus to overhang somewhat the tarsal claws and the latter are in fact hinged to the ventral edge of the tarsus. The tarsus itself is thicker than it is broad and does not taper evenly. This general arrangement of the tarsal structure again recalls that of *O. colubri*. In the present species the specimens which I have are sufficiently well preserved to make it possible to examine the 'eye-spot' structure on the pulvillus under a high power. Under an apochromatic $\frac{1}{12}$ in. objective with monochromatic light and critical illumination each structure is seen to be composed of a chitinized ring in which seem to be interspersed little granular rods, perhaps of a sensory nature. Inside the ring is supported an extremely thin and delicate membrane. A similar structure will probably be found in all the species which have this organ and it is probably tactile in function.

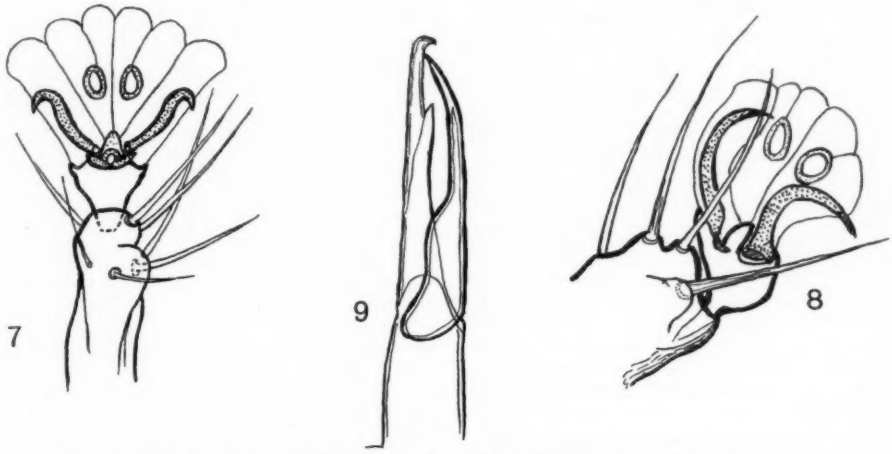

Fig. 7. *O. hammertoni* (Radford) female, claw and pulvillus of first tarsus. (A large stout hair overhangs the whole which is not shown here.)
Fig. 8. *O. hammertoni* female, lateral view of tarsus I.
Fig. 9. *O. hammertoni* female, right chela, dorsal view.

The chela of the female is more slender in my specimens as I have drawn it in Fig. 9. The apex of the movable digit is bent more obliquely than in the Fig. 13 of Radford and the apex of the fixed digit is turned so as to make a right angle with the shaft. The chelae, as well as the other structures described above, seem also to agree more with those of *O. colubri* than the original description would lead one to suppose and there seems to be little doubt that this species is fairly closely related to the latter. To bring the description into line with those of previously and subsequently described species I give the tarsal-tibial proportions for comparative

purposes. Tarsus I is 1⅓ times the length of tibia I. The tarsus of legs II and III is nearly twice that of the respective tibia and tarsus IV is nearly four times that of tibia IV. This last is a very distinct character of the species. Leg I is almost equal to leg II in length and leg IV is the longest.

My material consisted of two females; I have not seen the male.

A NOTE ON THE SPECIES *INCERTAE SEDIS* OF KEEGAN, 1943

Keegan in his paper gives a very interesting account of finding twenty-three specimens of an acarus very closely resembling *Entonyssus vitzhumi* and nine similar to *Ophiopneumicola colubri*, both from the same hosts as the type-specimens. Reading through the differences as tabulated by Keegan I have no doubt that these specimens belonged to the above-named species. The differences noticed in the form of the sternal and genital plates are accounted for by the fact that, as in most of the acarine internal parasites of other groups, the degree of chitinization is very variable and this often varies between parasites of different host individuals. Slight differences in the chelae mentioned by Keegan are, I find, often to be observed in different individuals of the same species and I believe that nearly all the species at present known will be found to be very variable.

It must be remembered too that much of the structure of these mites is very difficult to make out and it may be that authors have from time to time overlooked some of the smaller characters and it should not be assumed that when some such character is not mentioned by an author it is necessarily absent.

One point, however, in these descriptions cannot be explained in this manner, this is the presence of the 'eye-spot' structure on the pulvillus of the first pair of legs in the specimens of *O. colubri*, for this most noticeable feature is not to be found either in the original descriptions or figures and it would seem that in the type specimens it was absent. Until more observations are recorded and more is known of the life cycle of these mites it is impossible to know for certain what accounts for this discrepancy, but perhaps the most likely explanation is that the structure is developed in some stage of the life-history and aborts later. In any case, the claws and pulvillus of the first pair of legs seem to be very variable structures.

SUMMARY

All the Mesostigmatic mites which have been found as ecto- or endoparasites of snakes are listed and it is suggested that the latter are probably derived from the former. The homologies of the dorsal shields are established and a progressive fusion is shown to have taken place. On this evidence a new genus is erected for *O. bedfordi* (Radford) to include most probably the lung mites of African snakes. The life history and general biology are discussed. It is shown that there is no experimental evidence that these acari are vectors of disease or that they induce any change in the tissues of the host. Evidence is brought forward to show that the parasites of the more nearly related snake hosts are themselves more nearly related: some consequences of this are discussed. A key to the females of all the known lung mites of snakes is given together with the diagnosis of the new genus *Hammertonia* and of a new species, *Ophiopneumicola americana*. The view that the genus *Ophiopneumicola* is not a natural group is expressed. *O. hammertoni* Radford is redescribed and some points of significance discussed. The species *incertae sedis* of Keegan (1943) are discussed.

REFERENCES

Ewing, H. E. (1922). *Proc. U.S. Nat. Mus.* **62**, 1–26.
Ewing, H. E. (1924). *Proc. Ent. Soc. Wash.* **26**, 179.
Fonseca, F. da (1934). *Z. Parasitenk.* **6**, 508–27.
Fonseca, F. da (1940). *Mem. Inst. Butantan.* **14**, 53–8.
Hubbard, W. E. (1938). *Trans. Amer. Micr. Soc.* **57**, 400–6.
Hubbard, W. E. (1939). *Amer. Midl. Nat.* **21**, 657–62.
Hubbard, W. E. (1940). *Trans. Amer. Micr. Soc.* **59**, 243–7.
Keegan, H. L. (1943). *Parasitology*, **35**, 128–33.
Newstead, R. (1906). *Mem. Lpool. Sch. Trop. Med.* **18**, 45–50.
Radford, C. D. (1937). *North-West. Nat.* **12**, 38–42.
Radford, C. D. (1939). *Parasitology*, **31**, 243–54.
Sambon, L. W. (1913) in Boulenger, G. A. *The Snakes of Europe*, p. 108. Methuen.
Schmidt, F. L. (1940). *J. Parasit.* **26**, 309–13.
Stejneger, L. & Barbour, T. (1923). *A Check List of North American Amphibia and Reptiles*. 2nd ed. Cambridge: Harvard Univ. Press.
Vitzhum, H. G. (1931). 'Acari' in *Handbuch der Zoologie*. Krumbach & Kukenthal, **3**, Lief. 1. Leipzig: de Gruyter.
Vitzhum, H. G. (1935). *Z. Parasitenk.* **7**, 709–16.

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

SOME FURTHER ASPECTS OF ACTIVITY, SEASONAL AND DIURNAL

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(With 3 Figures in the Text)

CONTENTS

	PAGE
1. Introduction	27
2. Seasonal activity of the tick on different hosts	27
(i) Female ticks	27
(ii) Nymphs and larvae	27
(iii) Discussion	28
3. Further notes on seasonal activity differences between the tick stages	30
4. Diurnal activity	31
5. Summary	33
Appendix	33
References	33

1. INTRODUCTION

A previous paper (Milne, 1945*a*) deals with seasonal activity of *Ixodes ricinus* L. in Britain. The present paper extends these observations and adds a note on day-time activity as compared with night-time.

In Britain, the sheep is the sole host for which seasonal activity curves have been published. These curves are for female and nymphal stages. The only curves available for larvae have been derived not from an animal but from artificial blanket-dragging data (Milne, 1945*a*). The present seasonal activity studies were designed mainly to show whether blanket-larval curves are reliable and whether activity times for any stage differ on different hosts.

There is a fairly widespread belief among north England farmers that activity times differ in sheep and cattle. Some hold that although 'ticks' (probably females) are absent from their sheep in summer, they are present on their cattle at that season and even all the year round. Although unwilling to subscribe to this belief (for a number of reasons—see later), the writer could not deny the possibility of a host effect without examining curves for a number of different hosts sharing the same grazing. Unfortunately, he was unable to find a farm in north England where *handleable* cattle shared a heavily infested grazing with sheep. That part of the problem was therefore undertaken by workers in south Wales. Meanwhile he made weekly counts of ticks on sheep, rabbits (*Oryctolagus cuniculus* (L.)), skylarks (*Alauda arvensis arvensis* L.) and meadow-pipits (*Anthus pratensis* (L.)). The chosen wild animals were the most typical and most numerous representatives of the wild fauna sharing the ground with the sheep.

2. SEASONAL ACTIVITY OF THE TICK ON DIFFERENT HOSTS

This study was carried out on Lordenshaws farm in Northumberland. Here, ♀ tick activity has two phases: a high peak in spring and (usually) a much lower peak in autumn. For some unknown reason the autumn peak was almost suppressed in 1944 (see Fig. 1). Weekly counts of female ticks were made on ten Blackfaced ewes, and of all stages except males on ten rabbits and a varying number of skylarks and meadow-pipits. The Appendix shows the number of birds examined. The same sheep were used each week, but capture necessitated killing of the wild animals. Fig. 1 shows the activity curves for 1944, female ticks on rabbits being omitted from the graph but given in Table 1.

Note. Reliable nymphal counts could not be made on Blackfaced sheep chiefly because their attachment sites are mostly black in colour (see §3). Further, the sheep is too big a subject for reliable larval counts.

(i) *Female ticks*

On the small birds, female ticks were never found. On rabbits they were rare, but Table 1 shows that when they were present the time coincided with the few weeks around peak time for females on sheep. This suggests strongly that female ticks are active on sheep and rabbits at the same time.

Female ticks are much more numerous on larger mammals (see later paper), but *wild* mammals larger than the rabbit are unfortunately too scarce in north England to permit adequate weekly counts. In north-east Scotland, however, very dense populations of Scottish mountain or blue hare (*Lepus timidus scoticus* Hilzheimer) and red deer (*Cervus elaphus scoticus* Lönnberg) have been compared with sheep on the same or neighbouring pastures: Dr W. Moore states (private communication) that female activity is similar on deer, hares and sheep, the peaks being coincident. Therefore in four mammals the female activity curves are similar in form and timing.

(ii) *Nymphs and larvae*

The nymphal and larval activity curves on rabbits and small birds (Fig. 1) are for the most part

* Agricultural Research Council Unit of Insect Physiology.

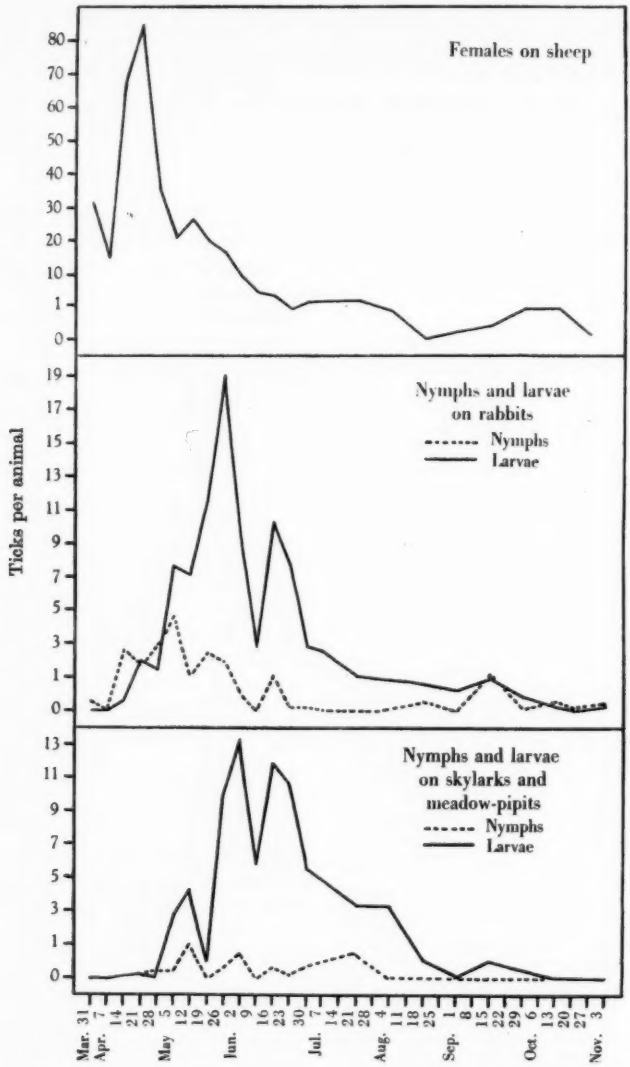

Fig. 1. Average tick activity curves during 1944 for Blackfaced sheep, rabbits, skylarks and meadow-pipits on Lordenshaws. (From weekly mean counts of total ticks on ten Blackfaced ewes, ten rabbits and a varying number—see Appendix—of skylarks and meadow-pipits.)

very similar in form. There is, however, a tendency for the bird curves to lag a little behind the rabbit curves. We are inclined to think this arises chiefly from smallness and irregularity in bird sample size, and hence that nymphal and larval activity is practically identical on rabbits, skylarks and meadow-pipits.

(iii) *Discussion*

We can imagine only two factors whereby a possible host effect on activity could result. The first is where conditions in the animal coat change during the year. In sheep, for example, grease

increases slightly in amount and diminishes in viscosity from early spring into summer (Milne, 1945*b*, 1946). No such change occurs in the cattle coat. Since grease can be lethal to ticks (Milne, 1945*b*), this *might* result in fewer live ticks being found on sheep than cattle in summer. Our *qualitative* comparisons of tick-infestation densities on cattle and sheep in summer do not, however, support this idea. We think that the farmer's impression arises from the fact that ticks are more easily seen on cattle from a few yards off and also the fact that he handles his cattle more than his sheep in summer. The second factor which could lead to a host effect is change of habit during the year; that is, any change tending to vary the amount of contact with vegetation. Such would be the case if an animal had at one time of year to cover less ground in search of food than at another time, owing to seasonal peaks in concentrations and abundance of, say, grass and insects. Again the breeding time partially or completely immobilizes one or both mates for a period. Thus several times during high tick activity a sitting pheasant or grouse has been found tick-free or with a very low infestation, while both birds have a relatively high susceptibility to infestation outside the nesting time. The slight tendency for activity in small birds to lag behind activity in rabbits (see Fig. 1), if it is real, could be explained as follows: as the season advances, rabbits need travel less because grass bulk is increasing, while birds, becoming more free of nesting ties, will be moving about more.

These factors would, of course, only affect seasonal activity on a particular animal species, not the ticks' time of readiness to feed on *any* suitable animal.

We cannot explain the following curious instance of hedgehog infestation (*Erinaceus europaeus* L.). In 1944 two hedgehogs were taken on Hethpool, one on 15 June and the other on 11 September.

Table 1. *Average female ticks per animal in weekly counts of ten sheep and ten rabbits sharing the same grazing*

1943			1944		
Date	Sheep	Rabbit	Date	Sheep	Rabbit
—	—	—	31 Mar.	31·3	0·0
9 Apr.	75·2	0·1	7 Apr.	14·8	0·0
16 „	106·9	0·2	14 „	66·5	0·1
23 „	69·0	1·7*	21 „	84·8	0·0
30 „	48·6	0·0*	28 „	36·0	0·1
7 May	47·0	0·7*	5 May	21·0	0·1
14 „	21·1	—	12 „	26·5	0·0
21 „	16·0	0·0	19 „	19·9	0·0
28 „	10·2	0·0	26 „	16·5	0·0

* Three rabbits only.

Table 2 shows that nymphal activity was still at its height on 13 June. The first animal yielded 13 nymphs and 94 larvae, the second 13 nymphs and 88 larvae (identification as *Ixodes ricinus* L. corroborated by Mr E. Browning of the British Museum). Now 5 years' intensive sampling on Hethpool by sheep and blanket counts (Milne, 1945*a*) has shown that tick activity is invariably close to zero after June. Further, in 1944, ten blanket drags taken two a day from 13 to 17 June on Hethpool yielded a total of 34 nymphs, i.e. 3·4 nymphs per drag; six drags on the same ground on 5, 11 and 17 September yielded 4 nymphs, i.e. 0·7 nymph per drag. Hence nymphal activity at the relevant times in June and September was in the approximate ratio of five to one according to the blanket. Superficial examination of sheep on 11 September confirmed the blanket findings. We are therefore left with the interesting question why the two hedgehogs had similar infestation weights. The difference between June and September blanket averages seems too great for the hedgehog results to have arisen from chance variation in extremely small samples, even though individual variation in tick counts is very high in sheep (Milne, 1943; and the following excerpt from unpublished data: total nymphs on muzzle ranged from 25 to 125 in ten sheep examined 9 April 1945).

Table 2. *Average standard tick counts on Hethpool sheep (hoggs) in* 1944

(The female peak was in early April.)

Date	Females	Nymphs
1 May	18·6	102·6
8 „	15·2	91·9
15 „	10·5	80·1
23 „	14·1	114·1
29 „	9·9	87·7
13 June	6·9	116·8

Apart from these considerations, the data for sheep, deer, hare, rabbit, skylark and meadow-pipit show no indication of any *marked* individual host effect on activity. Nor do additional bits and pieces of curves for other members of the wild fauna (not cited here). In general, activity on different hosts in the same area is identical and governed by some factors unconnected with host species.

3. FURTHER NOTES ON SEASONAL ACTIVITY DIFFERENCES BETWEEN THE TICK STAGES

'Males, females and nymphs have similar and concurrent activity curves in spring' (Milne, 1945*a*). Further data necessitate a slight modification of this statement.

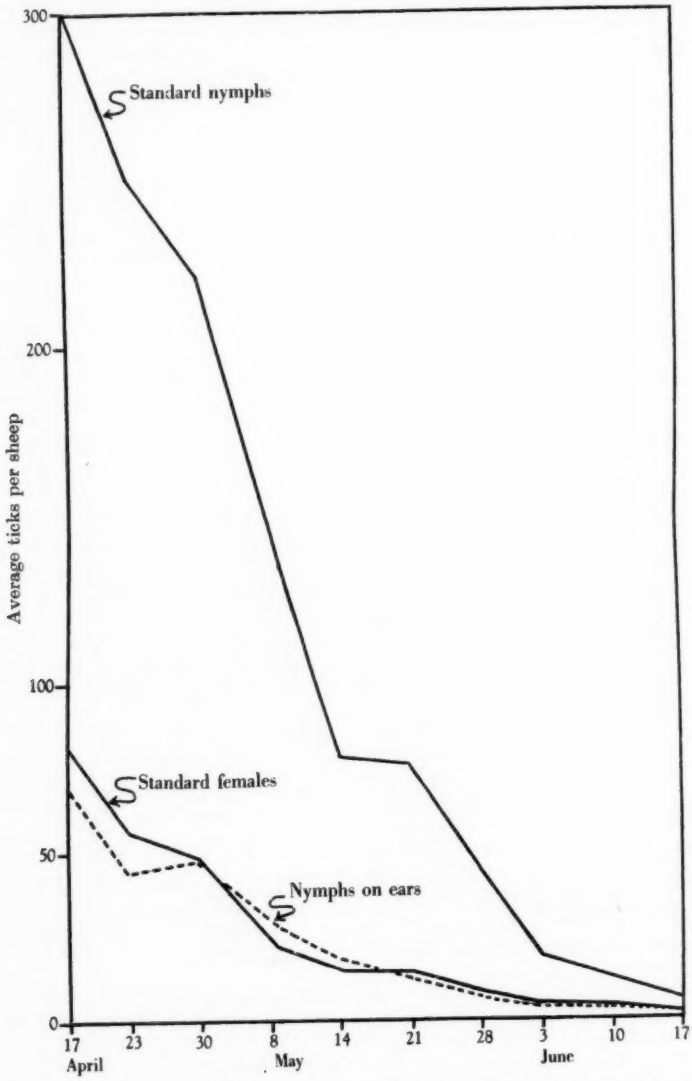

Fig. 2. Average standard weekly counts of female and nymphal ticks per Cheviot hogg on Hethpool. Average total of nymphs per sheep on (both) ears only (broken line) included to show how closely female and nymphal curves approximate to one another at similar infestation levels.

Both nymphs and females were counted weekly on the standard area (see Milne, 1943) of ten Cheviot hoggs (1-year-old-sheep) running on the same grazing at Hethpool farm, Northumberland. Nymphal counts are relatively easy on Cheviot sheep chiefly because the attachment sites are white while the nymphs are black. Fig. 2 shows the latter halves of the activity curves for 1945. These confirm the previous general finding, quoted above, that the bulk of female and nymphal activity is synchronous. This finding was a majority finding based on nine examples from counts on sheep (MacLeod, 1932, 1939) and three examples based on blanket data by the writer (Milne, 1945*a*). In MacLeod's work, nymphal and female peaks coincided in eight out of nine cases; in the writer's, in two out of three cases. The discrepancies were: nymphal peak 14 days later in one case (MacLeod, 1932, Chart 1) and 21 days later in one case (Milne, 1945*a*). MacLeod's discrepancy is dubious since another lot of sheep (N) on the same grazing at the same time did not show it and hence it might arise from sampling errors. The writer's one discrepancy cannot be so dismissed since such errors were avoided. It must be reconsidered here in the light of additional data in Table 2 and Fig. 1.

During 1944 a series of nymph-female standard counts (Table 2) were also made on Hethpool farm in the same circumstances as produced the data for 1945 (Fig. 2). Unfortunately the 1944 counts could not be started before 1 May nor continued after 13 June. Table 2 shows that during the 1944 season nymphal activity gave no sign of waning in unison with female activity within the period studied. This is corroborated by data from Lordenshaws in the same year: Fig. 1 shows the nymphal peak on rabbits to be two weeks later than the female peak on sheep.

MacLeod's data probably cover the years 1930–2. The writer's data cover 1941–5 (examples for two districts in 1944); in 1941, 1943 and 1945 nymphal and female activity was synchronous; in 1942 and 1944 the nymphal peak was 21 and 14 days respectively later than the female peak. That is two discrepancies in 5 years (or, including MacLeod's data, probably 8 years). The general statement should therefore now read: males, females and nymphs usually have similar and concurrent activity curves, but nymphs may occasionally have their peak 2–3 weeks later.

Fig. 1 also confirms the previous finding that the larval peak is several weeks later than the female peak. We now have data for 4 years, 1941–4, the first three being from blanket counts, the last from counts on rabbits and small birds. The larval peak was later than the female peak by 5 weeks in 1941, 4 weeks in 1942, 3 weeks in 1943 and 5–6 weeks in 1944. Shcheglova (1939) claims the opposite in Plyussa District, Leningrad Province, Russia: larval peak in May and nymphal peak in July; but the tables in his paper show that he has misled himself by mis-labelling his figures;* hence the nymphal peak is actually first as in England.

Considering 1941–4 only, the larval peak was at its latest relative to the female peak in 1941, but at its earliest in 1943. Nymphal peaks were later than female peaks both in 1942 and 1944. Hence there is no simple connexion between the data of larval peak one year and the consequent nymphal peak the next year. Until we know the lower thresholds of time-temperature for development of each tick stage in nature, further speculation is unprofitable.

4. DIURNAL ACTIVITY

Observations were made at Hethpool in 1944. During the night of 29–30 April, one drag on rough grass at midnight yielded 1 female tick, 44 nymphs and some larvae; a second drag at 3 a.m. yielded 14 nymphs and some larvae. This showed that ticks were active during the hours of darkness. In the afternoon of 28 April, two similar drags had yielded 12 nymphs, and 1 female plus 19 nymphs respectively; while on 2 May at the same time two drags yielded 9 nymphs and 7 nymphs. More detailed observations were then made during day and night on three occasions to see whether night activity was of the same order as day activity. As usual, 50-yard virgin stretches of plots were used for each drag. Equal numbers of 'simultaneous' drags (see Milne, 1943) were taken each time on the same rough grass and rough bracken plots, i.e. two drags on each plot except at the following times when three were taken: 3 p.m. on 12, 24 and 26 May, and 8 and 10 June. Since the activity curves for both kinds of plot followed one another fairly closely, the data for both have been lumped together. Fig. 3 shows the mean nymphs per drag together with the screen temperature on the plots. For the most part, the data for females (not given) bear out the data for nymphs. The blanket samples adult ticks less satisfactorily than nymphs (Milne, 1943).

The following additional meteorological data show that, except for one windy night, there were no abnormal differences between day and night weather during the periods of study:

12 *May, afternoon:* sunny; wind B2 (Beaufort number).

12–13 *May, night-time:* no moonlight; light cloud cover made it fairly dark throughout the night; wind B0–2.

24 *May, afternoon:* dull; wind B2, increasing towards night.

* C. Elton confirmed this as the author's, not the translator's, error.

24–25 *May, night-time:* no moonlight; mist around midnight but blown away by 1.30 a.m.; heavy clouds made it very dark throughout the night; wind B 5 from 9.30 p.m. to 1.30 a.m. when it increased to B 6 and remained so throughout the rest of the night; shower of rain at 7.30 a.m.

10 *June, afternoon:* sunny; wind B 3.

Fig. 3. clearly shows that, although ticks are active during the hours of darkness (i.e. on the vegetation tips ready to grab a host as they did the blanket), the activity-density then is considerably less than in daylight. Activity-density appears to follow screen temperature fairly closely. However, since the night temperatures recorded are not always below the level at which ticks are active in day-time (see Milne, 1945*a*), other factors such as diurnal temperature *range* and light intensity may also have some influence. Thus the night of 24–25

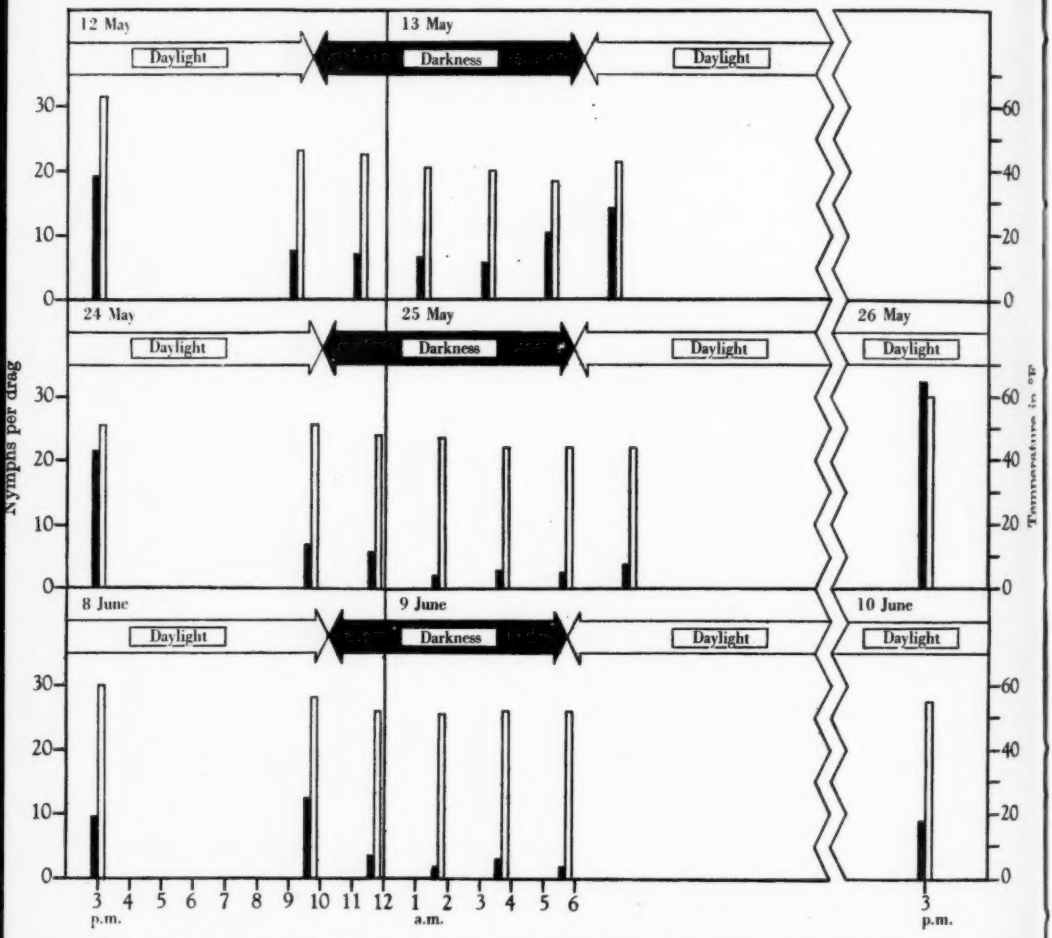

Fig. 3. Nymphal activity at intervals during daylight and darkness, and atmospheric temperature at the same times on a grazing at Hethpool. (Nymphal activity—black columns—estimated by blanket dragging. Temperature—white columns—from a thermograph in a screen on the grazing. Time equals Greenwich Mean Time plus 2 hr.)

26 *May, afternoon:* dull; 'soft' (i.e. warmish) wind B 5.

8 *June, afternoon:* sunny; wind B 2.

8–9 *June, night-time:* no moonlight; thick layer of cloud made it dark all night; wind B 1; vegetation very wet throughout the night.

May was 'very dark', of 8–9 June 'dark', and of 12–13 May 'fairly dark', i.e. a descending scale of darkness. On these nights the respective percentage reductions in nymphal activity (lowest level during dark upon highest level during previous day) were also on a descending scale, viz. 91, 85 and 71%. This suggests that the darker the night the less the activity. Unfortunately we have no data for moonlit nights. Finally we can not attribute the greatest activity depression (dark hours of 25 May) to high wind (B 5–6) alone since a very high activity occurred during daylight on 26 May with wind B 5. Temperature probably influences wind effect.

Activity during darkness will not profit the tick in the case of hill sheep since they lie up during the night, but will do so in the case of night-prowling wild mammals and birds.

5. SUMMARY

The idea has been mooted that the sheep tick (*Ixodes ricinus* L.) is active at different times on different host species. Tick-activity curves for sheep, deer, hare, rabbit, skylark and meadow-pipit are similar in form and timing. With these animals, therefore, there is no indication of any marked individual species effect on activity.

Where spring activity occurs, males, females and nymphs usually have similar and concurrent activity curves, but nymphs may occasionally have their peak 2–3 weeks later than adults. Larval activity is always later, the peak being several weeks after the female peak. The data for nymphs amend the general statement on activity in a previous paper by the present author.

Ticks are active during the hours of darkness but the activity-density then is considerably less than in daylight. This diurnal fluctuation in activity seems to follow fairly closely the diurnal fluctuation in atmospheric temperature.

The writer wishes to thank Mr T. P. McNicol, shepherds, farmers and gamekeepers who helped to make the collection of data possible; Dr W. Moore for data on deer and hares; also Dr V. B. Wigglesworth, F.R.S., Director, Agricultural Research Council Unit of Insect Physiology.

APPENDIX

The tick-activity curves in the birds were similar, hence lumped together in Fig. 1. Permission to collect these birds was granted by the Home Secretary for the larger purpose of assessing the parts played by farm stock and wild fauna in maintaining a tick population on hill pastures (see later paper).

Numbers of skylarks and meadow-pipits taken at Lordenshaws in 1944

Date	Skylark	Meadow-pipit	Total birds
31 Mar.	1	0	1
7 Apr.	2	1	3
21 „	3	7	10
28 „	1	4	5
5 May	1	4	5
12 „	1	3	4
19 „	1	5	6
26 „	1	3	4
2 June	4	5	9
9 „	2	3	5
16 „	4	5	9
23 „	4	6	10
30 „	1	3	4
21 July	3	0	3
4 Aug.	0	3	3
18 „	4	0	4
1 Sept.	3	0	3
15 „	0	2	2
13 Oct.	2	3	5
20 „	0	2	2
3 Nov.	0	2	2

REFERENCES

MACLEOD, J. (1932). *Parasitology*, **24**, 382.
MACLEOD, J. (1939). *Bull. Ent. Res.* **30**, 103.
MILNE, A. (1943). *Ann. Appl. Biol.* **30**, 240.
MILNE, A. (1945*a*). *Parasitology*, **36**, 142.
MILNE, A. (1945*b*). *Ann. Appl. Biol.*, **32**, 128.
MILNE, A. (1946). *Parasitology*, **38**, 34.
SHCHEGLOVA, A. I. (1939). *Problems of Ecology and Biocenology*, 5–6, 83. Leningrad. (In translation.)

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

THE INFESTATIONS OF HILL SHEEP

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(With 6 Figures in the Text)

CONTENTS

	PAGE
1. Introduction	34
2. Distribution of female ticks on the body	34
(i) Fore and hind regions: the relation of standard to total female tick counts	34
(ii) Head, axillary and inguinal regions	35
3. Zoning of different tick stages on the body	35
4. Female-tick infestations in the different age groups	37
5. Upper limits to female-tick infestation in hill sheep	38
6. The numbers of ticks fed by a flock of hill sheep	39
(i) Female ticks	39
(ii) Nymphal ticks	40
7. Sheep in good or poor condition	40
8. Individual female-tick infestations	40
(i) In any age group	40
(ii) The ewe and her own lamb	43
9. Physical, chemical and physiological differences between heavily and lightly infested sheep	43
(i) Differences in ten breeding sheep	44
(*a*) *p*H and yolk content of wool	44
(*b*) Wool characteristics	44
(*c*) Body dimensions	44
(ii) *p*H, grease and suint content of wool in ewes as compared with hoggs	45
(iii) Changes in *p*H, grease and suint content from spring into summer	46
10. Discussion and conclusions	46
11. Summary	49
References	50

1. INTRODUCTION

MacLeod showed (1932) that Blackfaced sheep were more susceptible to female-tick infestation than Cheviot sheep; and (1932, 1939*a*) that, within one breed, susceptibility decreased in the order: milk ewes, barren ewes, hoggs. The last named are sheep between 6 and 12 months old, i.e. about 1 year old during the spring tick season. Within any age group (ewe, hogg or lamb), female-tick infestations are highly variable among individuals grazing the same pasture (Milne, 1943). From slightly varying accounts by MacLeod (1932, 1939*b*), Moore (1938) and Cameron (1939), female-tick infestation sites would appear to be practically confined to the 'bare' or hairy areas or short wool adjoining these areas in sheep. Nevertheless, the writer (1945*b*) found as high as 38 % (average 16 %) of female ticks, on the anterior half of the body, actually attached under long wool. All this, however, leaves considerable gaps in our knowledge for ecological and control purposes. The present paper examines sheep infestations in greater detail, and chiefly as the necessary preliminary to a study of the relative parts played by sheep and wild fauna in maintaining tick populations on our hill pastures.

* Agricultural Research Council Unit of Insect Physiology.

2. DISTRIBUTION OF FEMALE TICKS ON THE BODY

(i) *Fore and hind regions: the relation of standard to total female tick counts*

Female ticks infest the hairy areas of sheep—head, ears and legs; the 'bare' areas—axillary and inguinal regions; and certain woolled areas—chiefly the short-woolled areas immediately bordering the bare or hairy areas though an appreciable proportion attach in the long wool of lower neck, chest and shoulders (see Introduction). Generally, female ticks are not found below the 'knee' joints (actually the ankle or wrist joints) on the legs, but an occasional tick is sometimes found as low as the hind fetlock. They are not found in the long wool of the withers, back, rump and flanks. In older sheep, they also infest the belly when the wool there is sparse and short—as it becomes with age. In lambs, with their short coat, female ticks occasionally occur on

back or sides, but as a rule the infestation pattern conforms to that of older sheep. A female tick is often found on the point of one or both teats in the ewe, sometimes hindering suckling. Very often a female tick is found on the tip of the docked tail.

Other tick stages occupy the same general regions as female ticks, but different zones of these regions (see § 3).

In comparing tick populations by counts of females on sheep, a *standard* count has been adopted (Milne, 1943). This includes all ticks on the fore-end of the body as far back as the posterior border of the axillae. A *total* count therefore includes the ticks on belly, inguinal region and hindlegs. Fig. 1 gives some idea of the relation between standard and total counts on Blackfaced sheep. On the totals from thirty-two observations between 31 March 1944 and 1 June 1945, about 85% of the female ticks attach in the standard region, i.e. forward of the posterior border of the axillae, and this proportion is remarkably steady when ticks are at all active. Thus when the standard average ticks per sheep is greater than 10 the mean ratio of total to standard is 1·177 and the range only 1·031–1·317; the corresponding figures for averages between 1 and 10 ticks are 1·158 and 1·000–1·600; and for averages less than 1, 1·238 and 1·000–2·000. This justifies the use of standard counts for comparisons. In general, standard count × 1·185 should approximate the total count at any time of year.

(ii) *Head, axillary and inguinal regions*

Table 1 shows that more female ticks attach on the head than on the axillary region, and more on the axillary than on the inguinal (groins). Since only a very small fraction of any region is ever utilized at one time by ticks, this taper distribution arises simply because the sheep walks head first, i.e. first come best served. Fig. 2 shows that the percentage of ticks on the head region increases slowly at first and then more rapidly with the advance of the season.

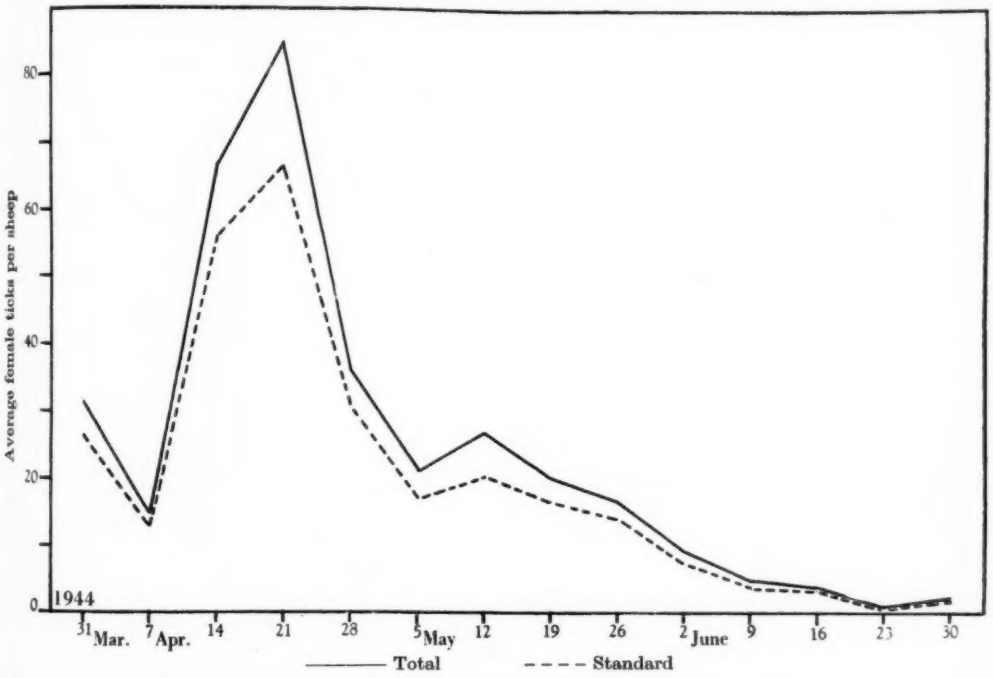

Fig. 1. Average standard and total female ticks per Blackfaced sheep on Lordenshaws. (From weekly counts on the same ten ewes. Section of 1944 data only.)

3. ZONING OF DIFFERENT TICK STAGES ON THE BODY

Although there is some overlapping, the different tick stages tend to attach in zones. We may illustrate

this by noting *main* distributions on head and axillary regions.

Head region

Female ticks: proximal part of ears (most numerous round the base), cheeks, under jaws, throat, and in short wool of neck.

Nymphs: distal part of ears, snout and chin.

Larvae: tips of ears, round nostrils and lips.

may suffice, e.g. positions towards the roots of the ears, or in bare parts of the axillae.

Another explanation of zoning has been furnished by a colleague, Dr A. D. Lees, of the Agricultural Research Council Unit of Insect Physiology. He suggests that females may require a more prolonged stimulation to make them attach than do nymphs, and nymphs likewise more than larvae. Thus females would have to travel farther from their

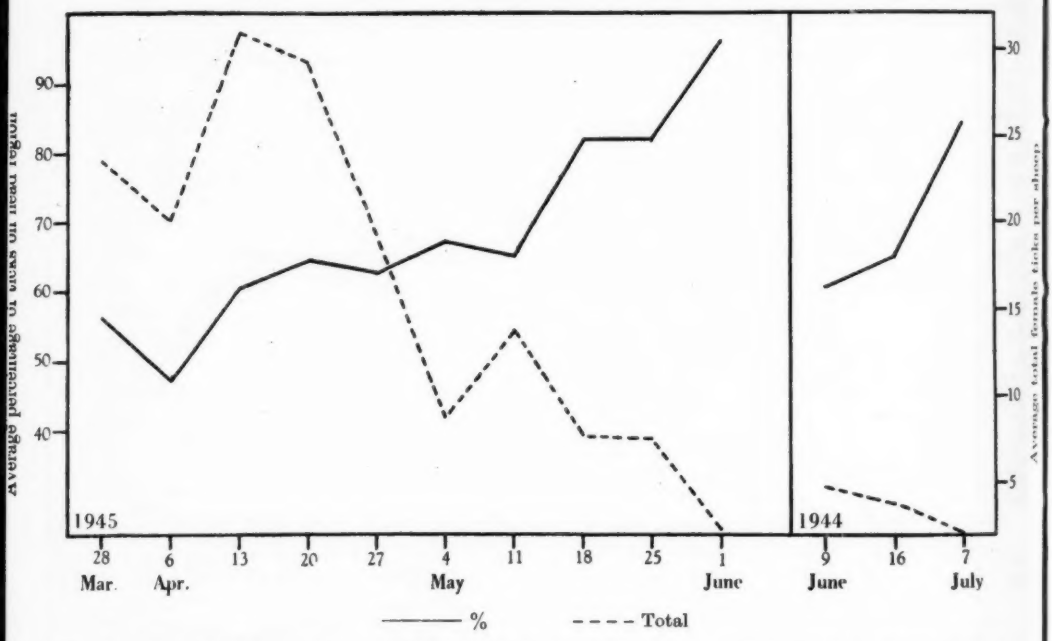

Fig. 2. Average total female ticks per Blackfaced sheep (broken line) and average percentage of these on head region (continuous line). (From weekly counts on the same ten sheep on Lordenshaws.)

Axillary region

Female ticks: upper arm, bare or hairy parts of axillae and short wool surrounding same.

Nymphs: lower arm.

Larvae: round fetlock.

Probably the majority of ticks on the fore-end of the body arrive via ears (distally), nose-lips and hoof-lower leg. Yet the larger the tick stage, the farther does its attachment site recede from these points. This recession may be correlated with gradients in friction. Thus on proceeding from lips back to cheeks, we find external friction decreasing and hair length (cover) increasing from about $\frac{1}{16}$ to 1 in. (0·16–2·54 cm.) or more in Blackfaced sheep; at the same time we meet first a zone of larvae, then a zone of nymphs, and finally a zone of females. Lessened external friction without increase in cover

Table 1. *Mean female tick counts from three regions of the sheep body*

(Same ten Blackfaced ewes used for each count.)

Date	Head	Axillary	Inguinal
1944: 9 June	2·89	1·00	0·89
16 ,,	2·44	0·78	0·55
7 July	1·78	0·22	0·11
1945: 28 Mar.	13·27	7·64	2·64
6 Apr.	9·50	7·60	3·00
13 ,,	18·70	9·40	2·80
20 ,,	18·80	9·50	0·90
27 ,,	12·00	5·90	1·30
4 May	5·90	2·40	0·50
11 ,,	9·00	2·40	2·40
18 ,,	6·30	1·10	0·30
25 ,,	6·20	1·10	0·30
1 June	2·20	0·10	0·00
Means for 1945 only	10·19	4·71	1·41

point of arrival on the sheep than nymphs, and nymphs farther than larvae, before becoming sufficiently stimulated.

4. FEMALE-TICK INFESTATIONS IN THE DIFFERENT AGE GROUPS

Fig. 3 illustrates part of the data in Table 2 which details average weekly total yields of female ticks per Blackfaced ewe, hogg and lamb on Lordenshaws, Northumberland, in 1944. The lambs belonged to the ewes under study. On the average, last must be due to the increasing activity and rapidly increasing size of the lamb. For example, 7–10-day-old Cheviot lambs may weigh 9 lb. in April, 31 lb. in June and 49 lb. in September. The hogg is also smaller than the ewe, but other factors may enter in (see Discussion).

Using standard counts (not given) instead of total counts (Table 2) the weekly ratios ewe : hogg from 31 March to 12 May inclusive were 1·252, 1·376, 0·989, 1·420, 1·604, 1·214 and 1·486; and from 19 May to 16 June they were 3·260, 2·622, 2·704,

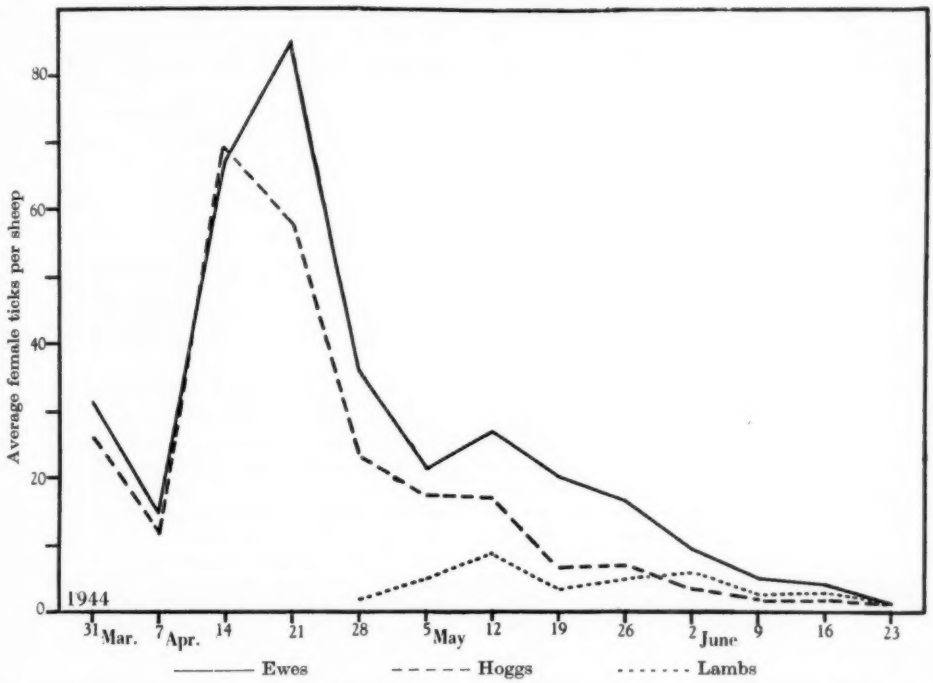

Fig. 3. Average female ticks per Blackfaced ewe, hogg and lamb running on one grazing at Lordenshaws. (Section of data in Table 2.)

each ewe fed 399·3 female ticks, each hogg 293·5 and each lamb 49·6 during the year, i.e. ewe: hogg: lamb :: 8 : 6 : 1 very nearly. After 26 May there was little difference between hoggs and lambs in the weekly counts, after 23 June, little difference between them and the ewes. That is, differences are not usually apparent at low-infestation levels (a fact having general application). Before 23 June, hoggs and lambs had consistently (one exception: hoggs, 14 April) a lower infestation than ewes, and the relation of ewe counts to hogg counts is fairly steady. From 28 April to 23 June, however, lamb infestation rose relatively to ewe infestation. This 3·250 and 2·462. Thus when average standard hogg counts were 10 or over, the equivalent ewe count was on the average 1·33 times greater; when hogg counts were between 1·0 and 9·9, the equivalent ewe count was 2·86 times greater.

Observations in Table 3 for 21 April 1944 (peak activity, see Fig. 1) bring out some interesting points. They were intended to test the idea sometimes put forward 'that most of the ticks on a sheep are attached to the ears'. With standard counts on Blackfaced sheep about 24 % of female ticks in ewes and about 44 % in hoggs were attached to the ears. 24 % of standard is equivalent to about 19 %

of total for ewes, and the like figure for hoggs is about 36%. Thus the claim quoted above is untenable for Blackfaced sheep.

Table 2. *Mean weekly total counts of female ticks on Blackfaced ewes, hoggs and lambs running on one grazing (Lordenshaws) in* 1944

(Same nine ewes with their nine lambs and same ten hoggs used each time in estimations. Lambs born between 15 and 18 April, therefore 7–10 days old on 28 April when counts began.)

Date	Ewes	Hoggs	Lambs
3 Mar.	0·0	0·0	—
10 ,,	7·8*	6·5*	—
17 ,,	15·6*	13·0*	—
24 ,,	23·4*	19·5*	—
31 ,,	31·3	25·9	—
7 Apr.	14·8	11·5	—
14 ,,	66·6	68·8	—
21 ,,	84·8	57·6	—
28 ,,	36·0	23·4	1·8
5 May	21·0	17·1	4·8
12 ,,	26·6	16·6	8·3
19 ,,	19·9	6·2	3·3
26 ,,	16·6	6·6	4·7
2 June	9·1	3·3	5·6
9 ,,	4·8	1·5	2·5
16 ,,	3·8	1·6	2·6
23 ,,	1·0	0·9	0·8
30 ,,	1·8	1·5	1·8
7 July	2·1	1·6	3·1
14 ,,	2·2*	1·7*	2·3*
21 ,,	2·4	1·9	1·4
28 ,,	1·6*	0·7*	1·2*
4 Aug.	0·8	0·5	1·0
11 ,,	0·4*	0·3*	0·6*
18 ,,	0·0	0·2	0·1
25 ,,	0·1*	0·3*	0·1*
1 Sept.	0·2	0·4	0·1
8 ,,	0·3*	0·7*	0·3*
15 ,,	0·4	1·0	0·5
22 ,,	0·6*	0·8*	0·8*
29 ,,	0·9	0·5	1·0
6 Oct.	0·9*	0·5*	0·6*
13 ,,	0·9	0·4	0·1
20 ,,	0·5*	0·3*	0·1*
27 ,,	0·1	0·2	0·1
Total	399·3	293·5	49·6

* Interpolations.

We saw above that during peak activity 24% of standard female ticks (i.e. all the ticks on head and axillary regions) in ewes were on the ears and 44% in hoggs. If we assume, reasonably, that ear counts are proportional to head-region counts, then the distribution of ticks on axillary and head regions is disproportionate in ewes and hoggs. Statistical analysis confirms this idea since, although there is no significant difference between ear means (Table 3), the difference between 'remainder of head plus axillary' means is significant, $P = 0{\cdot}02$ about. This is borne out in two out of four cases at low-level infestations (when differences tend to be hidden—see above) for which we have axillary and head-region counts for ten ewes and ten hoggs, viz.

Ratio: $\frac{\text{Axillary count}}{\text{Head count}}$

	Ewes	Hoggs
9 June 1944	0·3460	0·3333
16 June 1944	0·3197	0·0000
7 July 1944	0·1236	0·0832
11 May 1945	0·2667	0·2727

These data therefore indicate, though not with too great force, that the difference between ewe and hogg standard counts is probably due more to differences in axillary counts than head counts.

5. UPPER LIMITS TO FEMALE-TICK INFESTATION IN HILL SHEEP

At Crag farm, during spring peak activity, we have actually found a poor-conditioned ewe carrying a *total* infestation of about 500 female ticks. This is exceptional. The following approximations are based on 5 years of weekly counts on Hethpool and Crag and 3 years on Lordenshaws, together with less detailed observations on other hill farms in north England. As a rule, on *heavily infested* land stocked at one sheep to 1–5 acres, the highest weekly total count (i.e. spring peak) for a ewe is on the average 100–150 female ticks, while extreme upper limit of individual range is about 300. Further, on the average, a ewe feeds 500–750 female ticks in a spring season, i.e. about five times as many as she carries during the peak week. We may look on these approximations as upper limits to female-tick

Table 3. *Numbers of female ticks attached in different regions of individual Blackfaced ewes and hoggs at Lordenshaws,* 21 *April* 1944

Sheep	Region	Attached female ticks on individual sheep									Total	Mean
9 ewes	Ears	15	17	25	13	9	25	16	11	12	143	15·89
	Remainder of head plus axillary	18	25	91	67	66	37	83	46	27	460	51·11
	Inguinal	6	38	15	23	26	10	16	12	14	160	17·78
9 hoggs	Ears	11	18	9	15	26	33	22	31	23	188	20·89
	Remainder of head plus axillary	19	28	23	34	14	16	28	50	33	245	27·22

infestation in hill sheep for two reasons. First, because stocking densities greater than one sheep to one acre are impossible on hill land. Secondly, because the pastures studied have had practically the same sheep stocking for generations and have been infested for 50–100 years at least, i.e. their ground populations of ticks have attained their maxima so far as sheep-host availability is concerned.

6. THE NUMBERS OF TICKS FED BY A FLOCK OF HILL SHEEP

(i) *Female ticks*

From weekly average tick counts on each age group (e.g. Table 2) we may estimate the numbers of ticks fed per annum by the flock on a farm. Such estimates depend on several factors, viz.

(*a*) Sheep stock and proportions of age groups: Hill sheep stocks vary from one ewe or hogg to 1–14 acres, according to the carrying capacity of the grazing. Ideally the age groups should be in the proportions 4 ewes and 4 lambs to 1 hogg. Actually lambs may vary between 50 and 110 % of ewes at extremes, depending on the type of farm and the year (weather); lambs at 85 % of the ewe number is probably a fair average picture.

(*b*) The level of infestation: this seems to vary from year to year (Milne, 1945*a*).

(*c*) Whether there are one (spring) or two (spring and autumn) tick activity peaks per annum (Milne, 1945*a*). More ticks should be available in the latter case.

(*d*) Timing of the peaks: these vary from year to year (Milne, 1945*a*). This will have no effect on ewes and hoggs present on the grazing all the year round. But it will affect the numbers of ticks available to lambs. The latter always arrive at a fixed time; lambing begins at 12–15 April and is practically over 3 weeks later; and half of them (males) are sold off at a fixed time, August–October (depending on the custom of the farmer).

In the following, we choose typical examples of heavily infested farms in one- and two-peak districts of north England. The estimations are approximate but they illustrate the order of female tick numbers fed by sheep flocks.

Spring peak only

Lordenshaws, Northumberland, 1944. This farm of Blackfaced sheep was carrying about two-thirds of its normal stocking (reduced the previous year). Table 2 shows sheep to have fed female ticks on the average as follows: ewe, 399·3 ticks; hogg, 293·5; lamb, 49·6; i.e. 8 : 6 : 1 approximately. During the female activity season, 210 ewes, 90 hoggs and 229 lambs grazed the 1200 acres of the farm. Hence in 1944 the flock fed 121,627 female ticks—on the assumption that the engorgement period is 7 days. MacLeod (1932) gives the engorgement period range as '7–11 days, mostly 8–9'. With an 11-day period, the total for the flock becomes 77,399 female ticks ($\frac{7}{11}$ of 121,627). Thus on Lordenshaws in 1944, the sheep flock fed from 65 to 101 female ticks per acre, the higher figure being probably nearer the truth.

Hethpool, Northumberland, 1940. This farm of Cheviot sheep is normally stocked, but having better grazings, i.e. grass instead of heather, is about four times more heavily stocked than Lordenshaws. Data in a previous paper (Milne, 1945*a*) show that from 11 March to 18 November 1940, each hogg yielded on the average 414·9 female ticks in *standard* weekly counts. The 1940 peak at Hethpool occurred about the same time as the 1944 peak at Lordenshaws, and the activity curves were very similar; hence we may assume that ewe, hogg and lamb totals were also in the proportions 8 : 6 : 1. Using the factor given previously for converting standard to total counts, we estimate that the flock of 829 ewes, 209 hoggs and 710 lambs on this 1124-acre farm fed in 1940 between 448,281 and 704,442 female ticks, i.e. between 399 and 627 per acre.

At Hethpool in 1944 (Milne, 1946*a*), exhaustion blanketing of three 100 sq.yd. plots on the *lower* grazing slopes yielded female tick totals of 26, 9 and 22 each for the year, i.e. $19 \pm 5{\cdot}13$ per 100 sq.yd. or 678–1162 per acre. The majority of the tick population at Hethpool, however, is confined to the lower grazing slopes (Milne, 1946*a*), while our tick estimate for sheep includes the upper slopes. The proportions of female ticks fed by the wild fauna and missed by sheep or wild fauna are probably both small but included in the blanket results. Again, the ground population estimate is based on a rather small number of samples (plots) with a rather wide variation; and tick populations vary from year to year (Milne, 1945*a*). All things considered then we think that the estimated 399–627 per acre fed by sheep over the entire grazings in 1940 is of the right order.

Spring and autumn peak

Crag Farm, Cumberland, 1940–3. This farm of Herdwick end Swaledale sheep shares a common fell where one sheep (ewe or hogg) runs to every 5 acres and about 80 % of the ewes rear a lamb. Hence each 5 acres carry $\frac{4}{5}$ of a ewe, $\frac{1}{5}$ of a hogg and $\frac{16}{25}$ of a lamb. *Standard* weekly hogg counts over the entire annual activity gave the following numbers of female ticks fed per hogg per annum: 1940, 766·2; 1941, 709·0; 1942, 827·4; 1943, 846·7; mean, 787·3 female ticks per hogg. Transforming standard to total counts, taking ewe : hogg as 8 : 6, and lamb : hogg (arbitrarily) as 1 : 3 (because of autumn

peak and probably a slight underestimate), we calculate roughly that the sheep flock here feeds 175–275 female ticks per acre per annum.

An obvious suggestion from the above data is that the densest sheep stocking (Hethpool) results in the greatest number of ticks per acre fed by sheep.*

(ii) *Nymphal ticks*

It is extremely difficult to satisfy oneself that one has counted all the nymphs on a sheep. Cheviot hoggs were chosen as easiest subjects since they are white, short-woolled and relatively clean (the unfed nymph being only about 1 mm. in diameter and black). Both nymphs and females were counted weekly on the forebody (standard) of ten hoggs at Hethpool between 17 April and 17 June 1945 (Table 4).

Table 4. *Means of female and nymphal ticks per hogg from weekly standard counts on* 10 *Cheviot hoggs at Hethpool,* 1945

(Mean ratio, nymphs : females, is 4·660, standard deviation is ±0·9430 and standard error of mean is ±0·2982.)

Date	Females	Nymphs	Ratio Nymphs : Females
17 Apr.	81·2	300·4	3·699
23 „	56·1	251·0	4·474
30 „	48·3	221·4	4·584
8 May	21·4	134·6	6·290
14 „	14·3	78·4	5·483
21 „	14·2	75·4	5·310
28 „	8·3	44·6	5·373
3 June	4·4	18·6	4·227
10 „	3·8	12·0	3·158
17 „	1·4	5·6	4·000
Total	253·4	1142·0	

In 1945, nymphal and female activity curves were synchronous, and the ratio of nymphs to females on sheep varied remarkably little from week to week considering the high variation normal to tick counts in individuals. On the average 4·660 ± 0·298 nymphs were present for every one female in a weekly count. Now females gorge 7–11 days and nymphs 4–6 days (MacLeod, 1932). Using 7 and 4 days for females and nymphs respectively, then $4{\cdot}660 + \frac{3}{4} \times 4{\cdot}660 = 8{\cdot}155$ would approximate the true figure of nymphs fed by hoggs for every one female in the season. Taking engorgement periods of 11 and 6 days respectively, then $4{\cdot}660 + \frac{5}{6} \times 4{\cdot}660 = 8{\cdot}543$ would be the nymphal figure. These ratios may be slightly low due to missing nymphs in the counts.

A similar observation between 5 April and 13 June in 1944 gave 12·23–12·80 nymphs to one female fed by Cheviot hoggs. We must, however, discard this result since the nymphal peak was several weeks later than the female so that in half the data the nymphal numbers were still rising while the female were falling.* These data, of course, assume that nymphs and females are present in the same proportions on the hinder body as on the forebody.

We have no figures for larvae on sheep.

7. SHEEP IN GOOD OR POOR CONDITION

Ewes with lamb at foot are more highly tick-infested than barren ewes, or yearlings (hoggs). On the relatively poor hill grazings, lamb bearing and rearing is a heavy drain on the sheep system. The breeding ewe is generally in much poorer condition than the barren ewe or yearling. Condition in relation to tick infestation was therefore investigated among ewes. At the dates noted in Table 5, and with the help of a skilled shepherd, the leanest and fattest ewes were drawn from a flock of about 300 Cheviots running together on one hill grazing.

The terms 'fattest' and 'leanest' are relative. On hill grazings, few sheep attain the butcher's minimum standard of fatness. Hence the difference between fat and lean, though quite appreciable, was not very great and was least on 4 May 1942, which was a poor growing spring. Nevertheless, tick counts on the leanest ewes were significantly greater than those on the fattest ewes (see Table 5).

As a rule, the fleece of the fattest sheep is greasier (to touch and sight) than that of the leanest sheep, though there may be exceptions (see data 1941). Again sheep in poor condition are generally 'barer', i.e. sparser of wool, especially round axillary and inguinal regions and on belly, and the bare areas seem greater in extent.

Thus the better the condition of the sheep, the less bare and *apparently* the more greasy, and the lower the tick infestation.

8. INDIVIDUAL FEMALE-TICK INFESTATIONS

(i) *In any age group*

Over the years, the writer noticed that certain sheep tend to have high infestations and certain others low, in any age group. To see whether this was really consistent, four lots of ten sheep in three age groups (Blackfaced ewes, hoggs and lambs, and Cheviot hoggs) were examined individually with respect to their standard female tick infestations in 1944; one lot of ten sheep (Blackfaced ewes) were also examined in 1945. The findings were markedly similar in all five cases. Table 6 shows the data for

* This statement takes into account the fact that female tick population densities vary from year to year on any farm.

* Nevertheless, further observations show that the proportion of nymphs to females does vary from year to year.

Blackfaced hoggs, cited as typical. From a flock of ninety running together on one pasture, the ten hoggs were chosen at random, marked, and their individual standard female tick counts kept each week. In Table 6 the sheep are arranged in order of their totals for the period 31 March to 23 June 1944.

Table 6 shows that with individual counts we could have placed the female activity peak correctly in eight of the sheep and a week out (later) in two (nos. II and III). In 1945 another lot of ten sheep showed peaks ranging over 5 weeks, and only two of the sheep showed the true peak clearly; this is more usual and emphasizes individual variation from week to week.

Table 5. *Individual standard female tick counts on some of the fattest and leanest of* 300 *Cheviot ewes on one grazing at Hethpool*

Fattest	Leanest	Fattest	Leanest	Fattest	Leanest
—	—	—	—	1	3
—	—	—	—	5	4
—	—	—	—	1	2
—	—	—	—	3	2
—	—	—	—	1	5
0*†	—	—	—	4	0
1*†	5	—	—	4	5
2*	2	—	—	3	8
0*	9	—	—	3	6
4	2*	—	—	1	18
3	4	9	22	3	6
0	8	8	14	2	15
0*†	3	4	34	5	8
2*†	4	5	8	4	10
0*†	2	24	8	2	9
0*	4	9	14	1	17
0*	1	11	18	2	1
6‡	4	11	29	2	9
2‡	0*	23	32	4	7
1‡	1*	7	10	3	15
Mean 1·40	3·50	11·10	18·90	2·70	7·50
Probability 0·01		0·05 approx.		0·01	
Date 10 June 1941		4 May 1942		2 June 1942	

For 1941 only: * greasiest sheep, with a black oily material showing on wool and hair.
† barren.
‡ barest, i.e. less woolly generally and larger bare areas than usual under neck and between forelegs.

Table 6. *Individual standard counts of female ticks on ten Blackfaced hoggs running on one grazing at Lordenshaws,* 1944. *Sheep arranged in order (rank) of their totals for thirteen counts taken at weekly intervals*

	Sheep no.									
Date	I	II	III	IV	V	VI	VII	VIII	IX	X
31 Mar.	34	25	35	13	21	20	25	11	17	11
7 Apr.	12	2	14	8	14	10	6	7	8	12
14 ,,	83	55	46	72	53	48	49	53	45	42
21 ,,	49	81	50	56	39	46	32	49	40	30
28 ,,	22	22	25	23	20	24	23	17	9	7
5 May	23	16	18	11	23	14	15	8	8	4
12 ,,	24	11	23	9	15	13	14	14	10	3
19 ,,	9	2	5	2	6	3	8	6	4	4
26 ,,	10	8	6	2	3	4	3	4	7	6
2 June	9	5	1	2	1	0	0	4	3	2
9 ,,	2	3	1	3	0	1	1	1	0	0
16 ,,	1	3	3	0	0	0	3	1	2	0
23 ,,	2	2	0	0	1	0	1	0	1	0
Total	280	235	227	201	196	183	180	175	154	121

Obviously there is no consistent drop in counts from first to last sheep each week (Table 6). Considering the individual variation mentioned in the last paragraph (cp. also Milne, 1943), this cannot be expected, but Fig. 4 shows a distinct though irregular *downward trend* from first to last sheep when ticks are at all active, i.e. when highest count is 10 or over. From Table 6 we may also calculate that during the season any sheep had on the average a greater tick infestation than 62 % of the sheep below it in the series, and a tick infestation less than only 27 %; the remaining 11 % are accounted for by equalities. Omitting those weeks when the most highly infested sheep had fewer than 9 ticks (thus not permitting the possibility of a drop of one tick between each sheep), we find that 62 % rises to

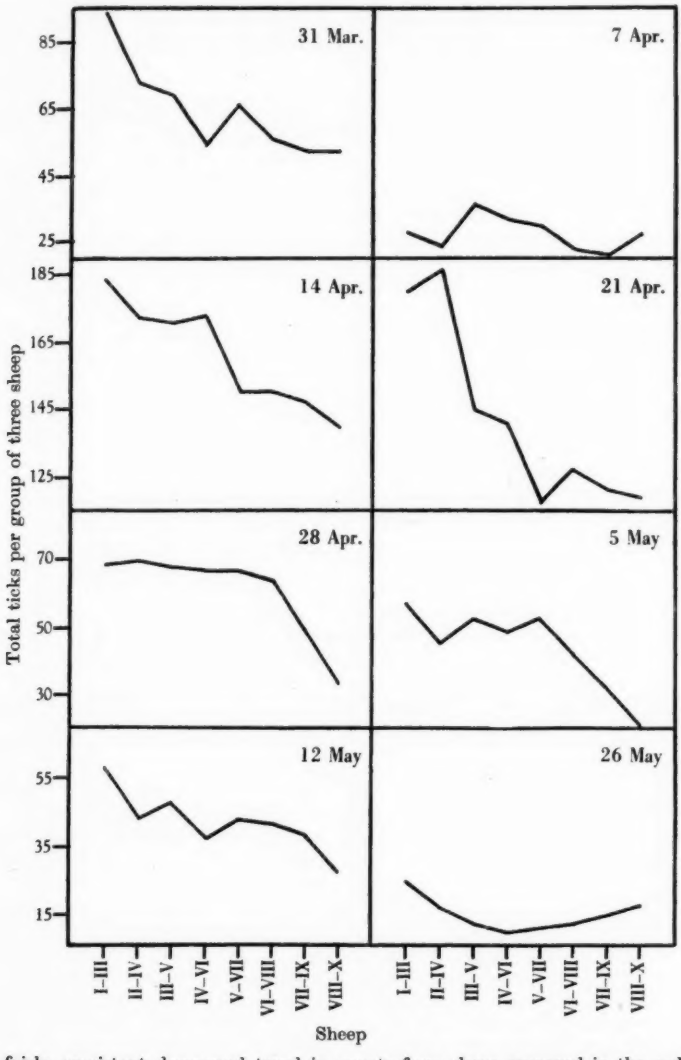

Fig. 4. Showing fairly consistent downward trend in counts from sheep arranged in the order (rank) of their seasonal female tick totals. (From those examples in Table 6 where the highest individual infestation is 10 ticks or over. Data calculated on the 'sliding the mean' principle, three at a time, in order to smooth the curves.)

66%. Again, sheep I exceeds sheep V in all but one of the thirteen counts (7 April); sheep V exceeds sheep X in all but two (26 May and 2 June): all three aberrations are very slight (see Table 6). Sheep X never exceeds sheep I. Clearly the farther apart in the series, the more consistently has one sheep a greater tick count than another. If the entire ninety sheep had been counted, there seems little doubt that individuals could be selected such that each has a consistently higher tick count than all the other selected sheep below. Thus, in general, sheep do show a tendency to consistent differences in weights of infestation.

Table 7

(*a*) Individual total counts of female ticks on Blackfaced ewes and their corresponding (own) lambs at Lordenshaws in 1944

Date	Sheep									
5 May	8 ewes	18	12	30	13	38	19	13	25	—
	8 lambs	1	3	3	6	10	6	2	7	—
12 May	9 ewes	32	32	11	17	34	30	48	21	14
	9 lambs	7	3	8	6	10	4	7	4	16
19 May	9 ewes	4	30	19	19	15	41	14	22	15
	9 lambs	2	1	2	2	3	5	4	6	5

(*b*) Mean per week of total female ticks on each of nine Blackfaced ewes and on each of their corresponding (own) lambs. Data cover ten weeks from 5 May to 7 July 1944

Mean per ewe	Mean of its lamb	Mean per ewe	Mean of its lamb
15·6	4·3	9·2	2·7
12·9	3·7	9·1	3·4
12·8	3·8	7·3	4·7
12·4	4·1	3·8	3·0
11·8	4·0		

Table 8

(*a*) Showing individual infestations with female ticks in two lots of five Blackfaced sheep in 1944 and 1945 and aberrations (*) from the 1944 order (rank) of infestation

Two age groups	5 sheep aged 1 year in 1944					5 sheep aged 2–5 years in 1944				
Total female ticks in 13 weekly standard counts from 31 Mar. to 23 June 1944	280	235	175	154	121	341	258	212	181	156
Total female ticks in 11 weekly total counts from 28 Mar. to 22 June 1945 (counts on 8 and 15 June being omitted)	263	141	98	221*	60	151*	282	197	120	107

(*b*) Showing order and infestations of these ten sheep in 1945. The ten sheep run on the same grazing at Lordenshaws

Individual sheep (2–6 yr. old)	1	2	3	4	5	6	7	8	9	10
Total female ticks in 11 weekly total counts from 28 Mar. to 22 June 1945	282	263	221	197	151	141	120	107	98	60

(ii) *The ewe and her own lamb*

Fig. 3 shows that average activity curves for ewes and lambs were practically parallel between 5 and 19 May 1944. Table 7*a* gives individual total female counts for ewe and corresponding lamb during this period. Correlation between lamb and ewe is 0·2612, and regression coefficient, lamb on ewe, is 0·0900, both highly insignificant statistically, probability being about 0·2. Table 7*b* gives mean per week for each ewe and corresponding lamb over 10 weeks. Extraction of means tends to iron out week to week variation in the individual. Again we find no evidence that highly infested ewes necessarily have highly infested lambs.

9. PHYSICAL, CHEMICAL AND PHYSIOLOGICAL DIFFERENCES BETWEEN HEAVILY AND LIGHTLY INFESTED SHEEP

At Lordenshaws in 1944, individual records of female tick counts were kept weekly for ten Blackfaced sheep running on the same grazing. Table 8*a* shows that but for two individuals the sheep kept to the same order (rank) of infestation in 1944 and 1945. The 1945 rank of infestation is shown in Table 8*b*; and in the present section the average bodily characteristics (factor *c* in § 10, below) of the

four or five less infested of these sheep are compared with those of the four or five more infested. In both groupings, i.e. fours or fives, the difference in average tick infestation is significant. Thus with sheep nos. 1, 2, 3, 5, 6, 7, 8 and 10, the mean infestation for the first four (229·3 ticks per sheep) is more than twice as great as the mean infestation for the last four (107·0). With sheep nos. 4 and 9 included, the first five (222·8 ticks) also have a mean infestation more than twice as great as the last five (105·2). A note is added on wool differences between ewes and hoggs; also a note on changes in *p*H, grease and suint content of wool from spring into summer.

greasy lock was taken from just behind each forelbow and bordering on the 'bare' axillae. Table 9 shows the data summary.

In no case did the data yield a statistically significant difference between means for more-infested and less-infested sheep. But consistently on both dates the more-infested sheep had slightly lower *p*H and higher grease and suint. These small differences, however, are unlikely to have any significant effect on the ticks.

(*b*) *Wool characteristics*

Samples of wool were taken from the middle of

Table 9. *Percentage grease and suint, and p*H, *in wool samples from four more-infested and four less-infested Blackfaced ewes. Percentages expressed on weight of raw wool after exposure for* 4 *days* (*minimum*) *to* 50 % *relative humidity. Each mean calculated from* (*a*) *sixteen values,* (*b*) *eight values. For details as to body sites of wool samples see text*

	Four more-infested ewes Nos. 1, 2, 3 and 5 in Table 8*b* Mean total of ticks per ewe = 229·3		Four less-infested ewes Nos. 6, 7, 8 and 10 in Table 8*b* Mean total of ticks per ewe = 107·0	
	Range	Mean	Range	Mean
		(*a*) 6 April 1945		
*p*H	6·59– 8·16	7·259	7·16– 7·77	7·429
Grease %	5·94– 9·05	7·300	4·56– 8·81	6·723
Suint %	6·46–12·82	9·565	6·52–11·71	9·407
		(*b*) 11 May 1945		
*p*H	6·46– 7·89	7·345	6·75– 7·87	7·409
Grease %	7·49– 9·89	8·970	6·58–10·40	8·710
Suint %	12·29–19·94	15·578	5·43–19·74	13·199

(i) *Differences in ten breeding sheep*

We have often noted unfed female ticks wandering in or over the fleeces of sheep (Milne, 1945*b*). Ticks not arriving on the sheep by legs or face must do this on proceeding to their attachment sites. Fleece characteristics, especially near the attachment sites, may therefore be of some importance.

(*a*) *p*H *and yolk content of wool*

Suint is the water-soluble matter in wool yolk, grease being the remainder. The Agricultural Chemistry Department of King's College, Newcastle-upon-Tyne, kindly undertook estimations from wool samples after methods of Hobson (1936, p. 853), except that *p*H was determined electrometrically. Wool samples were taken from sheep nos. 1, 2, 3, 5, and 6, 7, 8, 10 (Table 8*b*).

On 6 April 1945, four samples were taken from each sheep, one from each flank and one from each side of the chest, all as near the forelegs as possible without taking in the greasy locks immediately bordering the axillary region. On 11 May, one

the back, slightly to one side, in the ten sheep shown in Table 8*b*. Dr A. B. Wildman, of the Wool Industries Research Association, kindly classified these samples under seven heads. Table 10 is a summary of the data. In only one case was there a statistically significant difference, i.e. in the hair fraction (6). Here the less-infested sheep had coarser hair than the more-infested sheep. This accords well with our observation over the years that 'goat-skinned' sheep are least infested—all the more so since none of the ten sheep came near meriting the description 'goat-skinned'. Shepherds apply the term to those few members of the Blackfaced flock having a fleece that to some degree resembles in its coarseness the hairy skin of a goat. The coarser the hair in a sheep fleece then apparently the less susceptible is the sheep to infestation.

(*c*) *Body dimensions*

We may look on the sheep as a brush sweeping up ticks from the vegetation. The wider the brush and the closer its application, the more ticks should be swept up. But differences in widths of muzzle,

ear tips and body (across belly region) are negligible in a comparison of more- and less-infested sheep (Table 11). And although the difference between clearances of fleece tips of belly and sides from the ground is significant (P being just less than 0·05), it is the fleeces of the less-infested sheep which sweep closer to the ground. In Blackfaced sheep, the axillary and inguinal regions, including upper parts of legs are protected skirtwise by the fleece, but the head is not. Now Fig. 5 shows that sheep with fleeces sweeping closer to the ground tend to have a smaller percentage (35·4 as compared with 41·3) of their *total* ticks in axillary and inguinal regions. But this difference is too small to account for a 100% difference in total infestation weight. In short, fleece-tip clearance may have some small influence on total infestation of a sheep but would be a minor factor in infestation differences among individual sheep.

The more-infested sheep yielded slightly lighter fleeces (av. 3·1 lb. or 1·4 kg.) than the less-infested (av. 3·7 lb. or 1·7 kg.) at clipping in July. The difference was not, however, statistically significant, P being 0·3–0·4.

(ii) *p*H, *grease and suint content of wool in ewes as compared with hoggs*

Data in Table 12 reveal no significant difference between *p*H or grease content of wool samples from mid-back in Blackfaced ewes and hoggs (the num-

Table 10. *Means and ranges of wool characteristics from five more-infested and five less-infested Blackfaced ewes at Lordenshaws*, 1945

Characteristics	Five more-infested ewes Nos. 1–5 in Table 8*b* Mean ticks per sheep = 222·8		Five less-infested ewes Nos. 6–10 in Table 8*b* Mean ticks per sheep = 105·2		Probability (P)
	Range	Mean	Range	Mean	
(1) Max. crimped length of wool in cm.	6–10	7·80	8–11	9·60	Between 0·05 and 0·1
(2) Max. crimped total length of staple in cm.	19–24	21·20	21–25	23·40	0·1
(3) Hairiness: least hairy = 1, most hairy = 12	2–12	6·60	1–12	6·60	Means equal
(4) Density of undercoat (by touch): open = 1, dense = 5	1– 5	3·40	1– 4	2·80	Between 0·5 and 0·6
(5) Base of staple (includes wool undercoat):					
(*a*) 'Handle' (by touch): softest = 1, harshest = 6	1– 6	3·00	1– 6	3·00	Data equal in all respects
(*b*) Fineness (by sight): fine = 1, coarse = 3	1– 3	2·00	1– 3	1·60	Between 0·4 and 0·5
(6) Hair fraction:					
(*a*) 'Handle': very soft = 1, very harsh = 5	1– 3	2·00	2– 5	3·40	Just $< 0·05$, $t = 2·333$, $n = 8$
(*b*) Fineness: fine = 1, coarse = 5	1– 3	2·40	3– 5	3·60	Just $> 0·05$, $t = 2·121$, $n = 8$
(7) Kemp content: no kemp = 0, kempy = 6	0– 1	0·60	0– 6	2·00	Between 0·2 and 0·3

Table 11. *Some measurements (in cm.) of more-infested and less-infested ewes.* (*Same ewes as in Table* 9)

	Four more-infested sheep		Four less-infested sheep	
	Range	Mean	Range	Mean
(1) Muzzle width	6·9– 7·3	7·05	6·2– 7·1	6·70
(2) Ear tips width	27·0–29·5	28·38	28·0–31·5	29·38
(3) Greatest width of body (i.e. across belly and including wool)	54·0–62	58·00	55·0–68	59·25
(4) Clearance (i.e. height of fleece tips from ground)	8·0–13·5	10·75	5·0– 9·0	7·00

bers of sheep are small, however). But there is quite a significant difference in suint content, P being between 0·05 and 0·02. The hoggs had a little less than half the suint of ewes. Nevertheless this suint difference is probably not relevant to the present problem for the following reason: Two groups of ewes, with a much larger difference in tick infestation (Table 9) than that between hoggs and ewes (Table 2 and Fig. 3), showed no significant difference in suint content.

viscous in the warmer weather)'. Fig. 6 bears this out but the smallness of the increase from 28 March to 11 May, 2 % approx., and the subsequent irregularity, points to the probability that the first suggestion was prompted more by the change in viscosity than by the actual increase in grease. The thinner the grease, the greasier the fleece feels and the more easily do the hands become greasy. On the other hand, the large fall in pH, grease and suint on 1 June may be abnormal, due to abnormal

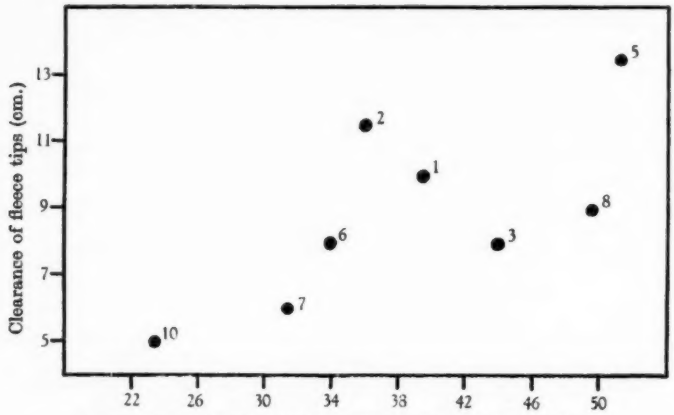

Fig. 5. Showing the relation between clearance (height of fleece tips of undersurface from ground) and percentage of total ticks in axillary plus inguinal regions of Blackfaced sheep. (Numbers beside dots show individual sheep as in Table 8*b*.)

Table 12. *Comparison of wool samples taken* 11 *May* 1945 *from mid-back (slightly to one side) of four Blackfaced ewes and four Blackfaced hoggs*

Sheep	pH	Mean pH	Grease %	Mean grease %	Suint %	Mean suint %
Ewe no. 5	6·69		8·69		8·31	
6	6·74		7·07		4·09	
8	6·82		8·06		10·32	
10	7·84	7·02	8·03	7·96	10·09	8·20
Hogg no. A	7·23		6·53		4·72	
B	6·82		5·88		3·90	
C	6·59		8·91		2·98	
D	7·14	6·95	7·50	7·21	3·87	3·87

(iii) *Changes in* pH, *grease and suint content from spring into summer*

Fig. 6 gives averages of pH, grease % and suint % in mid-back samples from four Blackfaced ewes at 3-week intervals. All three items vary, more or less together, from time to time. In another paper (Milne, 1945*b*) the writer, on the basis of the 'feel' of sheep in weekly handlings at tick counts, suggested that grease content 'increases from March into May as the pasture improves (and becomes less

rainfall just before (see Fig. 6). Certainly as rainfall decreased these values began to rise once more (22 June).

10. DISCUSSION AND CONCLUSIONS

We would imagine that the bigger the host, the more ground it would cover and hence the more ticks it would pick up. Size is obviously related to infestation weight in different age groups in sheep. We can

only compare lambs with older sheep from 28 April onwards, lambs being born about mid-April. In the period 28 April to 23 June (see Table 2), ewe, hogg and lamb fed on the average 138·8, 77·2 and 34·4 ticks respectively, i.e. in the ratio 4 : 2·25 : 1. Now the entire season's data (§4) showed ewe : hogg infestations to be as 4 : 3 which must be nearer the truth, since week to week variation will have less effect the longer the period. Body weights on 26 May, mid-point of period 28 April to 23 June, for ewe, hogg and lamb are of the order 100, 80 and 20 lb. respectively, i.e. 5 : 4 : 1, which is not very different from the infestation ratio 4 : 3 : 1. Thus infestation weight is directly related to size, though probably not quite linearly.

In any age group on one grazing, there is considerable range in infestation weights but individuals tend to keep their relative positions in this range throughout two successive spring seasons at least (§8). Thus infestation weight has an individual character. Since ground density of ticks varies over a hill pasture (with vegetational variation, Milne, 1944), three main factors should govern infestation weight. These are (*a*) choice of ground in grazing, (*b*) the amount of ground covered in unit time, and (*c*) physical and physiological characteristics of

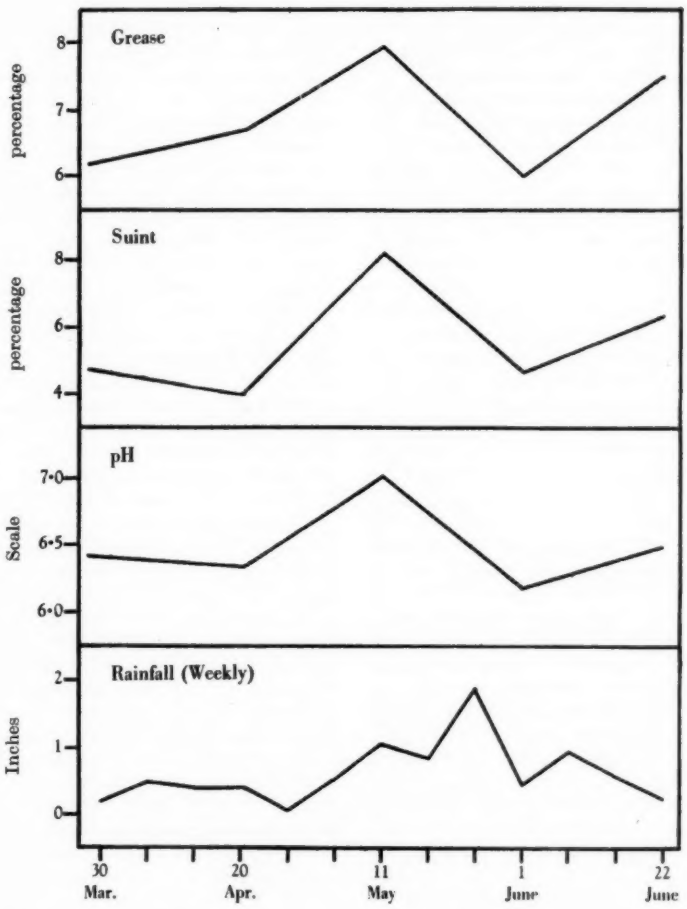

Fig. 6. Averages of *p*H, grease % and suint % in mid-back wool samples from four Blackfaced ewes at 3-week intervals; and total weekly rainfall at a meteorological station in the neighbourhood. (Sheep at Lordenshaws; rainfall at Cockle Park which is 10 miles away as the crow flies.)

individual body form and body surface in relation to their suitability for the tick.

Let us deal first with factor (*c*) which includes fleece characteristics, body dimensions, etc. The fleece varies widely in individuals of the same age group in hill sheep (Wildman, 1944). §7 showed that poorer-conditioned sheep had more ticks and *appeared* (touch and sight only) to be less greasy than better-conditioned sheep. MacLeod (1932) states: 'It is probable that the amount of grease in the wool affects the degree of infestation, dry-skinned unhealthy sheep offering more favourable conditions to ticks seeking attachment than sheep with a well-nourished, greasy skin and fleece.' The lethal effect of artificially filming attached ticks with wool grease (Milne, 1945*b*) seems to lend some support to this view. As the spring season advances, the percentage of ticks in the heavily fleeced areas decreases (Milne, 1945*b*); and the percentage of ticks on the head region increases relatively with the decrease in axillary and inguinal regions (§2). The head is much less subject to wool yolk (grease) than the other two regions since the wool comes little in contact with it and since it is less sheltered from external frictions and rain, etc. Now, Fig. 6 shows no very great increase in wool yolk with advancing season, but grease obviously becomes less viscous (no measurements). Ticks will then be more easily filmed with grease. Thus, if there is a grease effect, viscosity must be important in these instances.

There are two bits of evidence to set against the idea that grease is important. First, hoggs have relatively fewer ticks in the axillae than ewes (§4). We have no data for axilla greasiness in hoggs as compared with ewes, but there was no difference (except in suint content) in mid-back wool samples (§9). Secondly, no difference was found in grease and suint content, nor in *p*H, of wool samples from back, chest, flanks and axillae of two small groups of ewes *differing largely and significantly in tick infestation* (§9). These latter ewe groups differed very little, if at all, in condition, since each ewe had a lamb at foot. The point is that we may have large differences in individual infestation accompanied by no significant difference in greasiness.

We showed further (§9) that large differences of infestation in an age group could exist without significant difference in several other fleece characteristics and in various body widths. Indeed, there was remarkably little difference in physical and physiological characteristics of body form and body surface except in two instances, namely, that the less-infested ewes had coarser fleece hair and 3 or 4 cm. less fleece clearance from the ground.

Hair coarseness possibly has no direct connexion with infestation weight since ticks seem to be unhindered by the long coarse hair of Highland cattle or goats. On the other hand, coarse hair may render the fleece more difficult to penetrate in the presence of a close tangle of wool, i.e. the hair may have a stiffening effect on the staple.

Although fleece clearance seems to have some effect (Fig. 5), we showed (§9(*c*)) that *within an age group* of Blackfaces this effect was insufficient to account for more than a small part of the total difference in individual infestation. At the same time, although lesser fleece clearance appears to go with lesser infestation, the following contrary data might suggest we are wrong in linking these two things as cause and effect in any degree: Blackfaced sheep have very much less clearance than Cheviots but MacLeod (1932) found that Blackfaces were about twice as heavily infested as Cheviots going on the same grazing. Here, however, we are dealing with two very different hill breeds. Difference of infestation weight may be due to factors other than fleece clearance. For one thing, the Blackface fleece is much coarser; for another, shepherds hold that the Blackface is the more active animal, covering more ground in search of food (see below for implication of latter fact).

In studying factor (*c*) we omitted fleece humidity. Although we knew fleece humidities to be rather lower than atmospheric (see MacLeod (1940) for fleece humidities of *lowland* sheep; and Milne (1945*a*) for humidities at the vegetation tips, also unpublished data), we imagined, perhaps wrongly, that the tick would attach before low fleece humidity could become a danger. Once attached the tick gets its water from the blood. If fleece humidity varies *largely* within an age group, if a large proportion of ticks arrive via the fleece and if these take a long time to attach, then fleece humidity may play an important part in individual infestation difference. From our experience of physiological characteristics (above), however, we are inclined to think that fleece humidity will not vary sufficiently between individuals of the same age group for it to be an important limiting factor in itself.

On the whole, then, we suggest dismissing factor (*c*) as likely to be the *chief* circumstance in individual infestation differences within an age group, although it may contribute in a minor way.

We digress here to consider a broader aspect of the fleece: Although heavily fleeced areas do harbour a few engorging ticks (§§1 and 2), the 'bare' and hairy areas carry by far the majority. Further, dead unattached females are not infrequently found on or in the fleece (Milne, 1945*b*). Thus the fleece undoubtedly exerts some influence on infestation of sheep as a species. Probably the fleece has some hindering or lethal effect on ticks making primary contact with it instead of with the bare or hairy areas. This would explain why sheep with lesser fleece clearance have relatively fewer ticks in axillary-inguinal regions (§9). Less length of leg is

presented so more ticks will make primary contact with the *outside* of the fleece 'skirt' round the legs. The natural instinct of a hungry tick is to crawl upwards, as has often been observed with ticks picked up on the outsides of the trouser ends. Thus a tick arriving on the outside of the fleece 'skirt' may cruise fruitlessly up over the surface of the fleece, or, if the fleece is lethal, perish in trying to penetrate to the skin. Our colleague, Dr A. D. Lees, has made an interesting suggestion on the basis of his unpublished work. He says in a letter: 'As the unfed tick ages, its resistance to desiccation diminishes markedly; a proportion of the females finding a sheep host will be old individuals which will be particularly liable to desiccation if they happen to climb on at an inappropriate point (i.e. on the fleece). Not only is the humidity of the fleece fairly low but the temperature near the skin is high. They certainly would not survive more than a few hours of struggling in the fleece.'

Returning now to the remaining factors thought likely to govern infestation weight: (*a*) choice of ground in grazing, (*b*) amount of ground covered in unit time; §8 showed that the infestations of the ewe and her own lamb do *not* vary together. This fact enables us to weigh the importance of factors (*a*) and (*b*) since factor (*c*), although it probably contributes slightly towards absolute infestation weights in different individuals, would not influence any tendency for these weights to vary together. During its early months, the lamb is never very far from its mother. Hence the kind of ground covered by both must be very much the same (factor *a*). On the other hand, the amount of ground covered (factor *b*) by the lamb, although influenced to some extent by the mother's movements, differs in different lambs. Some are more active in their wandering and play round the mother-focus; others rest more between sucks. Thus we conclude that the amount of ground covered in unit time is very probably more important than choice of ground in determining individual infestation weight on a hill pasture. Indeed, we are inclined to think that the sheep's activity is the chief factor, but only the use of pedometers (if that were possible) could prove it. Within one age group 'breadth of front' presented to ticks on the vegetation varies little (§9) so that probably only the linear distance covered in unit time need be considered; but between age groups, breadth of front differs largely so that the area of ground covered becomes important, i.e. breadth of front × linear distance.

11. SUMMARY

In general, female ticks infest the hairy areas of sheep: head and legs; the (comparatively) bare areas: axillary and inguinal; and certain woolled areas: chiefly the short-woolled margins of the hairy and bare areas; they do not attach in the long wool of withers, back, rump and flanks.

More female ticks attach on the head than on the axillary region, and more on the axillary than on the inguinal region, obviously on the principle: first come best served.

Different tick stages tend to attach in different zones of the same regions; two theories are put forward to account for this.

On a hill pasture where the main tick activity occurs in spring only, the average totals of female ticks carried per annum by ewe, hogg and lamb were in the proportions 8 : 6 : 1. Host size is thought to be the chief governing factor.

Under existing British hill pastoral conditions, the upper limits to female tick infestation appear to be of the following order: average spring peak total count per ewe reaches 100–150, with extreme individual limit about 300; and about five times as many ticks are carried per ewe over the entire spring season.

On heavily infested hill pasture, stocked at one sheep to 1–5 acres, typical flocks feed from 100 to 600 female ticks per acre per annum. The densest sheep stocking results in the highest tick density.

For every one female tick, about eight nymphs were fed by sheep in one season.

The better the condition of a sheep, the less bare of wool and *apparently* (to touch and sight only) the more greasy, and the lower the tick infestation.

In any age group of sheep on one grazing, there is considerable range in weekly infestation weights but individuals tend to keep their relative positions consistently in this range. Sheep differing markedly in their infestation weights show a remarkable lack of difference in the majority of physical and physiological characteristics of body form and body surface. (Fleece humidity was omitted from study.) Notably, there is no indication of significant differences in fleece greasiness. Apparently only two characteristics go with the lighter infestations in Blackfaced sheep. These are coarser fleece hair and less clearance between fleece tips and ground, having doubtful and minor effects respectively.

The most important factor in individual differences of infestation within an age group is very probably individual activity, i.e. the more ground covered in unit time, the more ticks picked up.

The writer wishes to thank Mr T. P. McNicol, the shepherds and farmers who helped in this work; Dr A. B. Wildman of the Wool Industries Research Association for classifying wool samples; and Dr V. B. Wigglesworth, F.R.S., Director, Agricultural Research Council Unit of Insect Physiology, together with Dr A. D. Lees, also of the Unit.

REFERENCES

CAMERON, A. E. (1939). *Trans. Highl. Agric. Soc. Scot.* 1939 issue.

HOBSON, R. P. (1936). *Ann. Appl. Biol.* **23**, 852.

MACLEOD, J. (1932). *Parasitology*, **24**, 382.

MACLEOD, J. (1939*a*). *Bull. Ent. Res.* **30**, 103.

MACLEOD, J. (1939*b*). *Emp. J. Exp. Agric.* **7**, 97.

MACLEOD, J. (1940). *Ann. Appl. Biol.* **28**, 296.

MILNE, A. (1943). *Ann. Appl. Biol.* **30**, 240.

MILNE, A. (1944). *Parasitology*, **35**, 186.

MILNE, A. (1945*a*). *Parasitology*, **36**, 142.

MILNE, A. (1945*b*). *Ann. Appl. Biol.* **32**, 128.

MILNE, A. (1946*a*). *Parasitology*, **37**, 75.

MILNE, A. (1946*b*). *Parasitology*, **38**, 27.

MOORE, W. (1938). In *Report of the North East of Scotland Sheep Tick Committee.* Aberdeen.

WILDMAN, A. B. (1944). *Memorandum on the hill sheep of Scotland and their fleeces with special reference to the Scottish Mountain Blackface.* Wool Ind. Res. Ass., Leeds.

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THE LARVAE OF THE BRITISH SPECIES OF *PSYCHODA* (DIPTERA: PSYCHODIDAE)

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(With 84 Figures in the Text)

INTRODUCTION

In Britain the genus *Psychoda* Latr. contains sixteen species and two subspecies (Kloet & Hincks, 1945), the larvae of which occur in cow dung, decaying vegetation, and the bacteria beds of sewage purification works. A knowledge of these larvae would be useful both to the sewage biologist and the sanitary inspector, but for only three species have the larvae been fully described. Two of these, *Psychoda alternata* Say, and *Ps. cinerea* Banks, are inhabitants of the bacteria bed, and the larva of the former has been described on at least six occasions, the classical account being that of Dell (1905). The larva of *Ps. cinerea* has been described three times, the most complete account being that of Malloch (1917). The third *Psychoda* larva of which we have a full account is *Ps. albipennis* Zett. (Keilin & Tate, 1937), a species inhabiting dung and decaying vegetation. In addition to the foregoing there is a brief and inadequate description of the larva of *Ps. severini* Tonn. (Johnson, 1914), a species which is commonly present in the bacteria bed.

By collecting *Psychoda* flies in the field, and setting them up in culture, using scalded cow dung as a medium, the larvae of a further ten species have been obtained. It is the purpose of this paper to draw together the previous descriptions, and to describe those larvae hitherto unknown, with a view to filling this gap in our knowledge of the early stages of Diptera.

In order to discover something about the habitats of these larvae, likely breeding materials, such as cow dung, decaying vegetation, and mud from mud flats and ditch bottoms, have been collected and brought into the laboratory for the flies to emerge. The information obtained in this way has been supplemented by placing jars of scalded media, such as dung, rotting grass, and *Phormidium* (the algal growth from the surface of the bacteria beds) in localities where *Psychoda* abound, and leaving them there for a week. The jars have then been returned to the laboratory and the flies allowed to emerge. The information obtained from these observations has been summarized for each species and included in a short note following the description of the larva.

THE ANATOMY OF A TYPICAL *PSYCHODA* LARVA

A typical larva of the genus *Psychoda* is cylindrical, apodous, somewhat flattened dorsoventrally, and tapers slightly at both ends. It consists of a head and eleven body segments, of which the last bears the tubular respiratory siphon terminally and the anus ventrally. All segments except the last are subdivided into annuli, there being two of these in the three thoracic and first abdominal segments and three in each of the remainder. Each annulus may bear a sclerotized, strap-like dorsal plate, and the number and arrangement of these offer diagnostic characters of great value. One may be present on each annulus, when they total twenty-six, or they may be confined to the posterior two annuli of each segment, making twenty in all. In some species the more anterior annuli lack plates so that their number is less than twenty, and may be as low as two.

The larva is amphipneustic and the anterior spiracles are paired cylindrical structures borne on the lateral margins of the posterior annulus of the first segment. The posterior spiracles are borne at the tip of the sclerotized, tubular, tapering siphon, and lie between the bases of a dorsal and a ventral pair of processes. The body of the siphon is divisible into two regions, a heavily sclerotized, proximal region, which may bear some sort of microcuticular ornamentation such as spines or hairs, and a lightly sclerotized distal region through which the paired tracheae can be seen by transparency. At, or near, the junction of these two regions is a sensilla of unknown function but of characteristic and specific form; it will be known as the siphonal sensilla.

The ventral pair of terminal processes is the larger. Each process consists of a sclerotized cylindrical base and an unsclerotized apical portion on which are from one to four semicircular annular thickenings. The tip of the process carries a ring of radiating fringed setae and one or two sensory processes. The spiracles are paired and each consists of a circular opening partially occluded by a plug which is connected to the surrounding rim by a number of radiating threads. A similar central plug and radiating threads can be seen in the anterior spiracle (Fig. 14). The head is strongly sclerotized and bears a pair of lateral ocelli. The occurrence of

these ocelli is not constant, and larvae both with and without them have been reared from the egg batch of a single female. They will not, therefore, be used as a source of diagnostic characters. The epicranial suture is U-shaped and the arrangement of the setae bordering it offers characters useful in the separation of certain species. The antenna is a small mushroom-shaped structure, around the base of which a ring of sensory processes is grouped. The mouth opens on the ventral surface and the mouth parts are complex. The labrum is recurved and spiny and overhangs the buccal cavity. The mandible consists of a basal portion bearing a lightly sclerotized protuberance on which are two long setae. On the body of the mandible is a large palmate tooth which is opposed to a smaller simple tooth. Between the mandibles lie the premandibles, a pair of simple triangular sclerites having toothed posterior margins. The maxilla is oblong in shape and bears a group of sensory processes anteriorly and a group of inwardly directed teeth posteriorly. Medially is a single conical tooth mounted on a papilla. The mentum is a simple transverse plate bearing the labium which consists of a group of sensory processes borne on a slight prominence. The great uniformity of the mouth parts of these larvae, and their relative inaccessibility renders them unsuitable as sources of diagnostic characters. They have been described in detail for *Ps. albipennis* by Keilin & Tate (1937), and in less detail for *Ps. alternata* by Haseman (1910). Lloyd, Graham & Reynoldson (1940) have described the mandible and premandible of *Ps. alternata* and *Ps. severini*. Very similar mouth parts occur in related Psychodid genera and Feuerborn (1933) has described those of *Maruina indica* whilst Zavattari (1924) has described those of *Telmatoscopus meridionalis*.

Each body segment bears regularly arranged setae which conform to a definite plan throughout the genus, and Feuerborn (1927*a*) has worked out the details of their arrangement for *Ps. alternata*. Though their arrangement differs little from species to species their degree of development differs widely. In some species they are long and robust, reaching to the segment behind, whereas in others they are so small as to be quite hidden in the integumental covering of microtrichial elements.

These microtrichial elements show a considerable variety of structure, and may take the form of hairs, spines, combs, or plaques. Usually some of these elements are specialized on the ventral surface of the abdominal segments, forming ambulatory areas, where they are larger and more heavily sclerotized. On the thorax the elements are subdivided, taking the form of minute hairs which together give the appearance of a fine soft pile. The covering of microtrichial elements will be referred to as the vestiture.

THE PREPARATION AND EXAMINATION OF *PSYCHODA* LARVAE

In the identification of *Psychoda* larvae great use is made both of the number of dorsal plates and of the vestiture, and in some species these details are only visible after staining. As a general rule if the larva has dorsal plates on each segment it will only be necessary to clear the specimen. The caustic potash-glacial acetic acid-clove oil technique is excellent for this purpose. If, however, the larva is of the type with dorsal plates missing from some of the anterior segments it should be given a light boiling in caustic potash, stained in carbolfuchsine, dehydrated in alcohol, and cleared in carbol-xylol. This will bring out the very lightly sclerotized elements in the vestiture which would otherwise be invisible.

A further character used in the separation of these larvae is the proportion between the length and width of the siphon. For the calculation of this ratio the width is measured at the level of the siphonal sensilla, and the length is measured as the distance from the bases of the terminal appendages to the commencement of sclerotization at the base of the siphon. This ratio is not absolutely constant for each species, and can only be taken as a general guide.

The total length of the larva is also included in the description of each species but little reliance can be placed on this measurement since it is dependent on the temperature at which the larva has been reared, the size decreasing with increasing temperature. The data given apply to averages of ten larvae reared between 15° and 20° C. It is rarely possible to know the temperature prevailing during larval life with specimens from the field and size is affected by other factors such as food shortage and overcrowding. Hence, this measurement has little diagnostic value.

KEY TO THE LARVAE OF THE BRITISH SPECIES OF *PSYCHODA*

Section A

Two dorsal plates on each of the first four segments and three on each of the remaining six, making twenty-six plates in all.

Section B

Some plates missing from the anterior segments so that the number is less than twenty.

Section C

Two plates present on each segment, making twenty plates in all.

SECTION A

1, (2). Two oval sclerotized plates present on the ventral surface of segment ten, in addition to the full series of twenty-six dorsal plates. *Ps. surcoufi* Tonn.
2, (1). No plates present on the ventral surface of segment ten. 3
3, (4). Vestiture consisting of numerous toothed plaques, siphon six times as long as broad. *Ps. severini* Tonn.
4, (3). Vestiture consisting of hair-like elements. 5
5, (6). Anterior dorsal plate of segment four very much reduced, not more than one-quarter to one-fifth as wide as the plate in front, sometimes absent altogether, anterior spiracle mamillate. *Ps. cinerea* Banks
6, (5). Anterior dorsal plate of segment four not so reduced, about half the width of the plate in front, anterior spiracle cylindrical. *Ps. gemina* Eaton

SECTION B

7, (8). More than two dorsal plates present. 9
8, (7). Only the last two dorsal plates of the series present, situated on the posterior two annuli of segment ten. 16
9, (12). Third dorsal plate of segments eight and nine bearing two or three strong, heavily sclerotized, backwardly directed thorns at their posterior angles. 10
10, (11). Nine to eleven dorsal plates; third plate of segments eight and nine bearing thorns, that of segment ten without thorns, or, if present, very small; vestiture of long, single, flame-like hairs. *Ps. phalaenoides* L. Subsp. *elongata* Tonn.
11, (10). Nine dorsal plates; thorns present at the posterior angles of the third dorsal plate of segment ten, as well as segments eight and nine; vestiture of plaques with spiny fringes. *Ps. crassipenis* Tonn.
12, (9). Third dorsal plate of segments eight and nine (if present), lacking sclerotized thorns. 13
13, (14). Number of dorsal plates variable, usually seven clearly visible, sometimes some fainter and more lightly sclerotized plates visible in front of these; plates strap-like, three to four times as long as broad; siphonal sensilla rod-like, not terminating in a hair; vestiture of plaques with spiny fringes. *Ps. albipennis* Zett.
14, (13). Larva vermiform, elongate, with six small, oval dorsal plates on segments nine and ten, sometimes with three minute spot-like plates present on segment eight as well; siphonal sensilla terminating in a hair; vestiture of fan-shaped plaques bearing unequal spiny teeth. *Ps. alternata* Say
15, (14). Six dorsal plates present, one on each of the posterior two annuli of segments eight, nine and ten; siphonal sensilla rod-like with no terminal hair; vestiture hairy. *Ps. lobata* Tonn.
16, (17, 18). Siphon short and evenly tapered from base to tip, five times as long as broad, vestiture of coarse shaggy hair, ambulatory areas indistinct, five setae bordering epicranial suture. *Ps. trinodulosa* Tonn.
17, (16, 18). Siphon consisting of a distal parallel-sided portion joining an expanded base, six times as long as broad; vestiture hairy, very fine and short, ambulatory areas forming distinct crescents of stronger spines on segments four to ten; four setae bordering epicranial suture. *Ps. grisescens* Tonn.
18, (17, 16). Siphon as above only seven times as long as broad; vestiture hairy, moderately fine and short, ambulatory areas ill-defined; five setae bordering epicranial suture. *Ps. setigera* Tonn.

SECTION C

19, (20). Siphon fully formed, six times as long as broad; dorsal plates without a posterior border of spines; lateral body setae borne on small sclerotized circular plates. *Ps. spreta* Tonn.
20, (19). Siphon reduced to a minute conical structure borne at the end of an expanded semicircular eleventh segment; anterior dorsal plate of segments four to ten bearing a posterior fringe of seven or eight long, backwardly directed spines. *Ps. brevicornis* Tonn.

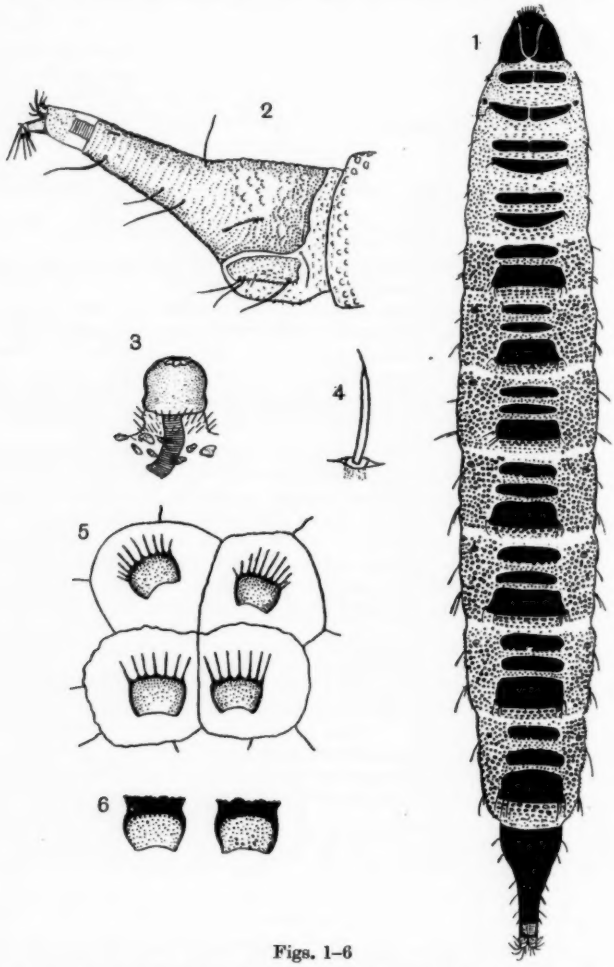
SECTION A

Psychoda surcoufi Tonn.

Larva (Fig. 1), narrow, cylindrical, 5·3 mm. long. Each annulus with a dorsal plate, making twenty-six in all, median dorsal plates showing no signs of reduction; an additional pair of oval plates present ventrally on segment ten; body setae prominent. Vestiture consisting of closely-set plaques bearing posterior fringes of spines (Fig. 5); plaques subdivided into minute elements on dorsum of thorax and behind each dorsal plate; segments four to ten

bearing two rows of characteristic ambulatory scales ventrally (Fig. 6); each scale exhibiting a pronounced sclerotized posterior margin; similar scales present dorsolaterally on two small papillae on each side of each intersegmental line in segments five to ten, and as a fringe bordering the posterior margin of the anterior dorsal plate of these segments. Siphon (Fig. 2), seven times as long as broad consisting of a small expanded base and a long parallel-sided distal portion; ventral processes each with a single annular thickening; siphonal sensilla (Fig. 4), parallel-sided, curved, terminating in a short hair. Anterior spiracle (Fig. 3), as tall as wide, swollen at the base.

The larva of this species was twice found in cow dung collected in November, 1943, from two localities near Leeds. In the laboratory it bred readily on all the media tried (dung, decaying leaves, decaying hay, and *Phormidium*).



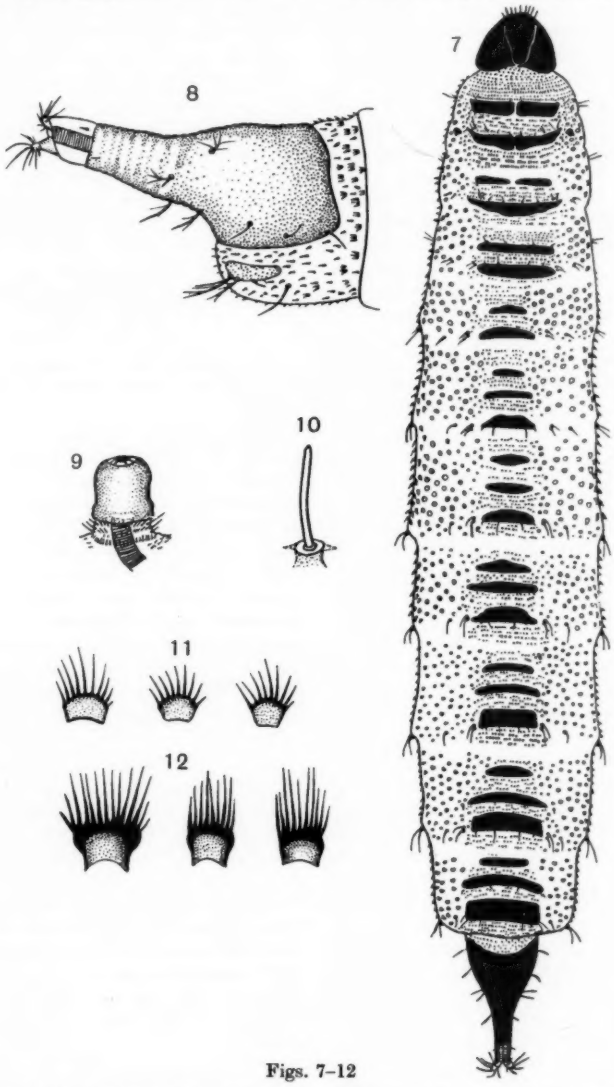
Figs. 1–6

Fig. 1. Larva of *Psychoda surcoufi* Tonn. × 33. Fig. 2. Siphon × 69. Fig. 3. Anterior spiracle × 420. Fig. 4. Siphonal sensilla × 590. Fig. 5. Vestitural elements of dorsum of abdomen × 590. Fig. 6. Ambulatory elements × 590.

Psychoda severini Tonn.
subsp. *parthenogenetica* Tonn.

The larva of this species has been described by Johnson (1914). The author mistakenly supposed

that it was the larva of *Ps. sexpunctata* Curtis = (*alternata* Say) he was describing, but both the text and the appended photograph show that *Ps.* In addition to this account, the mandible and pre-mandible have been described and figured by Lloyd, Graham & Reynoldson (1940).



Figs. 7–12

Fig. 7. Larva of *Psychoda severini* Tonn. subsp. *parthenogenetica* Tonn. × 33. Fig. 8. Siphon × 69. Fig. 9. Anterior spiracle × 425. Fig. 10. Siphonal sensilla × 585. Fig. 11. Vestitural elements of dorsum of abdomen × 585. Fig. 12. Ambulatory elements × 585.

severini Tonn. subsp. *parthenogenetica* Tonn. was the species concerned. The material was collected from a sewage filter where both these species occurred, and this would account for the confusion.

Larva (Fig. 7), cylindrical, rather flattened, 4·2 mm. long.

Each annulus bearing a dorsal plate, making twenty-six in all, median dorsal plates somewhat

reduced; setae of body conspicuous, the lateral groups on the posterior annuli of segments four to ten set on prominent papillae. Vestiture of strongly sclerotized plaques, each bearing a posterior fringe of hairs (Fig. 11); elements subdivided on thorax and behind each dorsal plate; ambulatory elements (Fig. 12), similar to those of general body surface but with longer fringes, forming parallel rows on the ventral surface of segments four to ten. Siphon (Fig. 8), six times as long as broad, tapering from the expanded base to the tip; ventral processes each bearing two annular thickenings; siphonal sensilla (Fig. 10), parallel-sided, bluntly pointed, lacking a terminal hair, inserted on the distal, lightly sclerotized, region of the siphon. Anterior spiracle, (Fig. 9) cylindrical, taller than wide, showing irregular annular thickenings around the base.

The larva of *Ps. severini* is commonly found in the bacteria beds of sewage purification works but it is very catholic in its choice of breeding grounds and almost any moist decaying vegetable matter will serve as food. It has been recorded from rotten carrots, rotten mangolds, chicken dung, horse and cow dung, the refuse left in disused farm feeding troughs, household drains, and mud flats (Lloyd, 1945). It does not appear to be a regular inhabitant of the field deposit of dung as it has only emerged occasionally from the eighty-nine samples followed through.

Psychoda cinerea Banks

The larva of this species was first described by Haseman (1908), under the name of *Ps. domestica*. Muttkowski (1915) gave a more detailed description of this larva but believed that *Ps. cinerea* and *Ps. domestica* were separate species. Malloch (1917) pointed out the synonymy of the two species and redescribed the larva. Of these three accounts the last is the most concise, but it does not serve to distinguish the larva from that of the twin species, *Ps. gemina* Eaton.

Larva (Fig. 13), cylindrical, slightly flattened, 5 mm. long.

Each annulus bearing a dorsal plate, making twenty-six in all; plates of median segments much reduced, those of segment four spot-like; anterior plate of segment four one-quarter to one-fifth the width of the plate in front, occasionally absent; body setae conspicuous. Vestiture consisting dorsally, of long hairs terminating in two or three points (Fig. 17); a region of close-set single and double hairs present behind each dorsal plate and forming an extensive area on the thorax; ambulatory areas consisting of branched comb-like hairs (Fig. 18), present on the first two annuli of segments four to ten. Siphon (Fig. 16) five times as long as broad, tapering from the swollen base to the tip; ventral processes bearing very long radiating setae and four annular thickenings; siphonal sensilla rod-like, terminating in a hair; unique (except for *Ps. gemina*) in possessing a long seta inserted adjacent to it (Fig. 19). Anterior spiracle cylindrical, almost twice as long as broad, narrowing halfway up to give it a mamillate appearance (Figs. 14, 15), basal region showing two or three irregular rings of short annular thickenings.

The larva of this species is sometimes an inhabitant of the sewage bacteria bed though not as regularly as are *Ps. alternata* and *Ps. severini*. Lloyd (1945) records it in association with these two species in a mud flat, and it has been found in household drains. It does not, however, appear to inhabit the field deposit of dung as none has emerged from any of the samples followed through.

Psychoda gemina Eaton

Larva (Fig. 20), cylindrical, slightly flattened, 3·5 mm. long, very similar to that of the foregoing species.

Each annulus bearing a dorsal plate, making twenty-six in all, median plates somewhat reduced, anterior plate of segment four, rectangular, not less than half the width of the plate in front; body setae of moderate length. Vestiture consisting dorsally of short, comb-like hairs with five or six points (Fig. 24); a region of close-set single and double spines present behind each dorsal plate and forming extensive areas on the thorax; ambulatory areas consisting of short-fringed combs (Fig. 25) present on the first two annuli of segments four to ten. Siphon (Fig. 21), very short, three to four times as long as broad, consisting of a swollen base and an abbreviated tubular portion; ventral processes bearing long radiating setae and four annular thickenings; siphonal sensilla short, rod-like, terminating in a hair; a long seta inserted adjacent to it (Fig. 23). Anterior spiracle, (Fig. 22) cylindrical, 1–1½ times as tall as wide, of even diameter from base to tip, lacking basal annular thickenings.

The larva of this species occurs in the decaying mass of leaves and mud found in ditch bottoms, and adults emerged on two occasions from samples of this material taken to the laboratory. Like *Ps. cinerea*, this species has never emerged from samples of field dung.

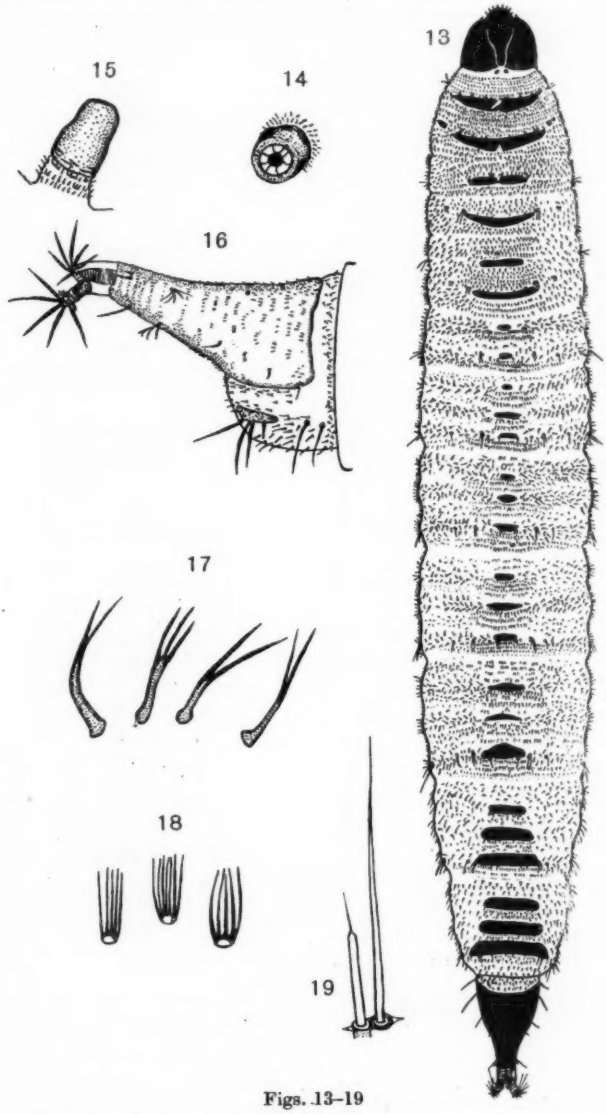
Section B

Psychoda phalaenoides L. subsp. *elongata* Tonn.

Larva (Fig. 26), cylindrical, slightly flattened, 3·7 mm. long.

Dorsal plates present on each annulus of segments eight, nine and ten, making nine in all; vestiges of plates sometimes present on the two posterior annuli of segment seven; two or three backwardly directed, sclerotized thorns present at the posterior angles of the third plate of segments

eight and nine, absent, or, if present, very small, on segment ten, (Figs. 27, 28): body setae short and inconspicuous. Vestiture consisting dorsally of a covering of single and double flame-like spines set on expanded, quadrate bases (Fig. 31); ambulatory areas consisting of comb-like elements (Fig. 32), forming ill-defined areas on the ventral surface; thoracic modification most pronounced on prothorax, progressively less marked on meso- and metathorax. Siphon (Fig. 29), rather variable in length, four to six times as long as broad, tapering evenly from the base to the tip; ventral processes



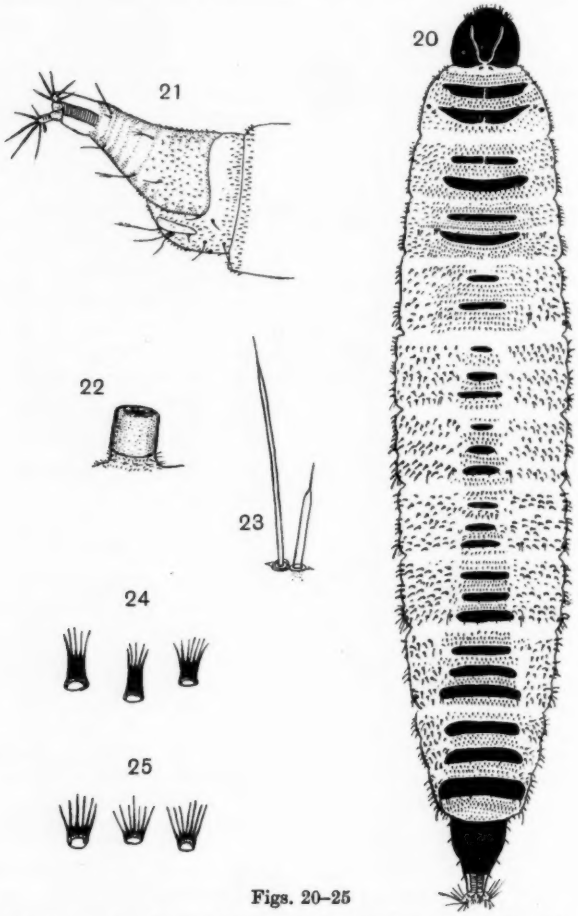
Figs. 13–19

Fig. 13. Larva of *Psychoda cinerea* Banks × 33. Fig. 14. Anterior spiracle, dorsal view × 425. Fig. 15. Lateral view × 425. Fig. 16. Siphon × 69. Fig. 17. Vestitural elements of dorsum of abdomen × 585. Fig. 18. Ambulatory elements × 585. Fig. 19. Siphonal sensilla × 585.

bearing a single annular thickening; siphonal sensilla short, rod-like and pointed. Anterior spiracle, cylindrical, domed apically, slightly taller than wide (Fig. 30).

This larva has frequently been found in the field dung deposit where it is often exceedingly abundant.

Dorsal plates present on each annulus of segments eight, nine and ten, making nine in all; two or three robust sclerotized thorns present on the posterior angles of the third plate of segment ten as well as of segments eight and nine (Figs. 34 and 35); body setae short and inconspicuous. Vestiture



Figs. 20–25

Fig. 20. Larva of *Psychoda gemina* Eaton × 33. Fig. 21. Siphon × 69. Fig. 22. Anterior spiracle × 420. Fig. 23. Siphonal sensilla × 590. Fig. 24. Vestitural elements of dorsum of abdomen × 590. Fig. 25. Ambulatory elements × 590.

One deposit (collected 20 October 1943, near Leeds) yielded 1156 flies in the course of the following week. It has been recorded from no other breeding material.

Psychoda crassipenis Tonn.

Larva (Fig. 33), cylindrical, slightly flattened, 3·2 mm. long, very similar to that of *Ps. phalaenoides*.

consisting of a uniform covering of sclerotized plaques of varying form; elliptical and possessing a posterior fringe dorsally (Fig. 39); irregular and lacking a fringe bordering the dorsal plates (Fig. 38); shield-shaped and possessing a strong, spiny fringe ventrally (Fig. 40); ambulatory areas indefinite; thoracic modification most pronounced on the prothorax, progressively less marked on the meso- and metathorax. Siphon (Fig. 36), six times

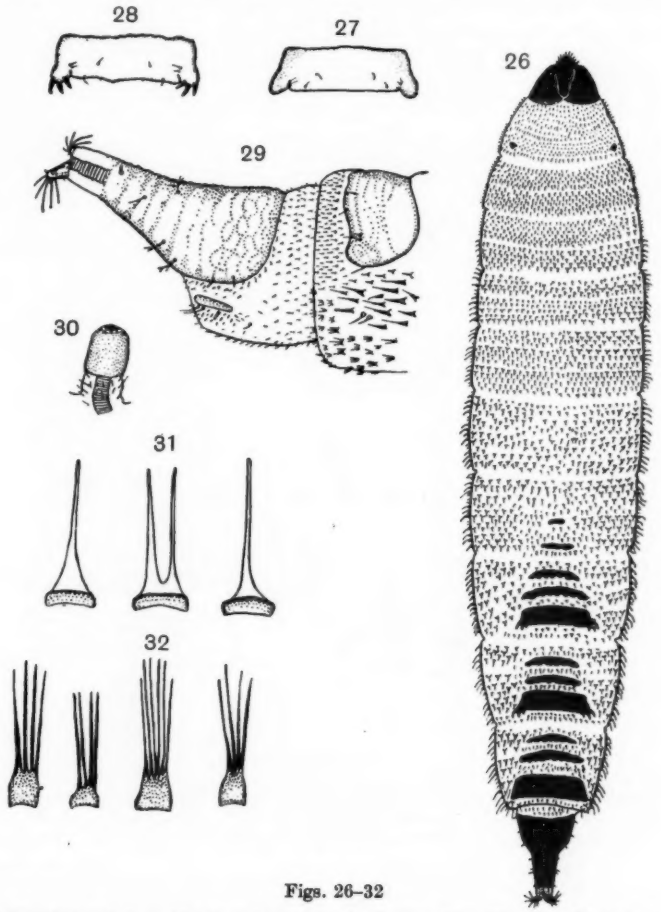
as long as broad, terminal portion slightly tapering, broadening out abruptly to the swollen base; ventral processes bearing a single annular thickening; siphonal sensilla short, rod-like and pointed. Anterior spiracle globular, taller than wide, of maximum diameter medially (Fig. 37).

The larva of this species was found in a deposit of cow-dung by Dr Ll. Lloyd near Appleby in August 1943, and this is the only definite record of its habitats. The adults have been taken regularly on trees surrounding a dung heap near Leeds.

paper includes an account of the mouth parts of the larva.

Larva (Fig. 41), cylindrical, slightly flattened, 3·7 mm. long.

Dorsal plates rather variable in number; usually the posterior seven or eight of the series are visible; sometimes additional plates are visible anteriorly; plates strap-like, three to four times as broad as long, the posterior angles not bearing teeth; body setae of moderate length. Vestiture of flattened comb-like elements (Fig. 45), lateral elements stouter and projecting from the surface; ambulatory areas present ventrally on the first annulus of segments four to ten, consisting of three or four rows of plate-like elements bearing strong, spiny



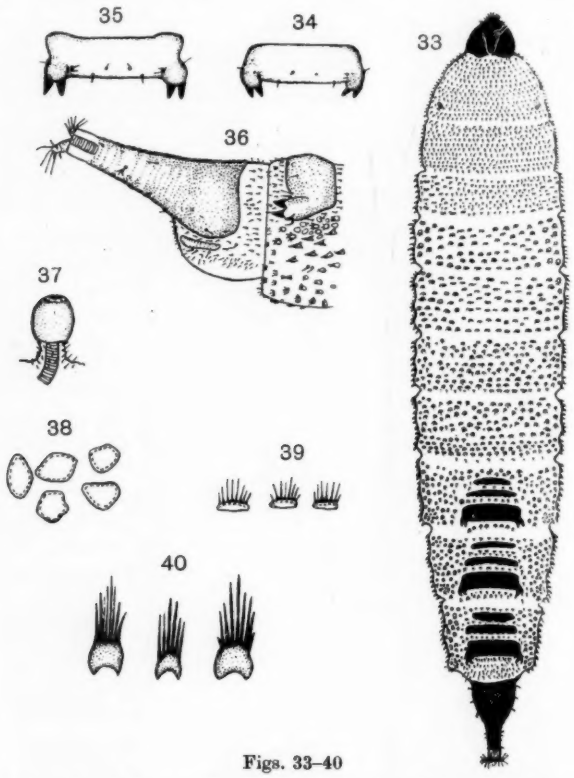
Figs. 26–32

Fig. 26. Larva of *Psychoda phalaenoides* L. subsp. *elongata* Tonn. × 33. Fig. 27. Third dorsal plate of segment ten × 69. Fig. 28. Third dorsal plate of segment nine × 69. Fig. 29. Siphon × 69. Fig. 30. Anterior spiracle × 420. Fig. 31. Vestitural elements of dorsum of abdomen × 590. Fig. 32. Ambulatory elements × 590.

Psychoda albipennis Zett.

The larva of *Ps. albipennis* Zett. has been described in detail by Keilin & Tate (1937), and the

fringes (Fig. 46). Siphon (Fig. 42), slender, seven to eight times as long as broad, tapering from the expanded base to the tip; ventral processes bearing a single annular thickening; siphonal sensilla (Fig. 44), short, rod-like and bluntly pointed. Anterior spiracle (Fig. 43), protuberant, wider apically than at the base, mounted on a prominent papilla.



Figs. 33–40

Fig. 33. Larva of *Psychoda crassipenis* Tonn. × 33. Fig. 34. Third dorsal plate of segment ten × 69. Fig. 35. Third dorsal plate of segment nine × 69. Fig. 36. Siphon × 69. Fig. 37. Anterior spiracle × 420. Fig. 38. Vestitural elements bordering lateral margins of dorsal plates. Fig. 39. Dorsal elements. Fig. 40. Ventral elements, all × 590.

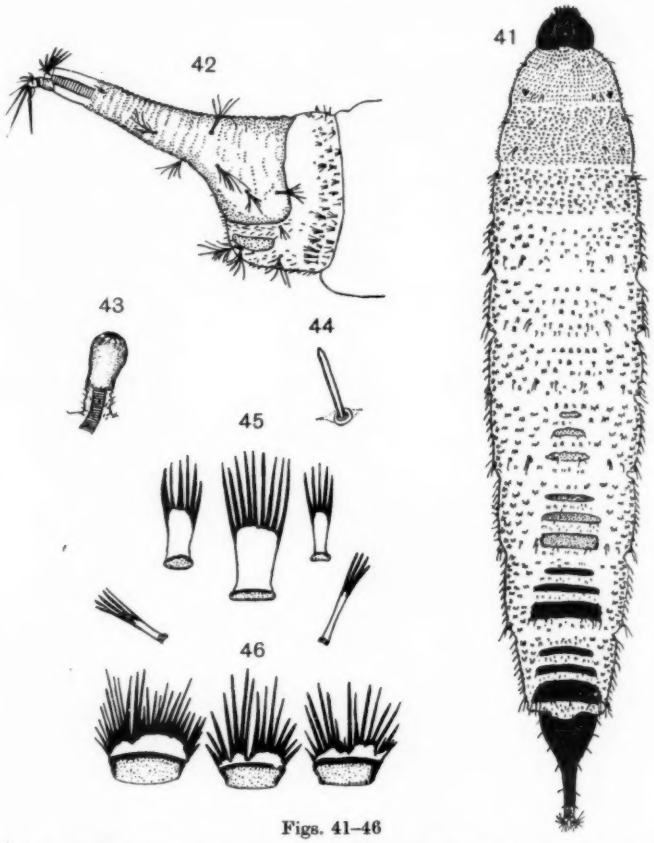
This species has been found breeding in horse dung, in rotting carrots and occasionally in cow dung. It is one of the commoner species of *Psychoda* and its relative scarcity amongst the flies emerging from the dung samples indicates that this is not its usual habitat.

Psychoda alternata Say

This is the best known of all the *Psychoda* larvae for not only has it been described several times, but it has been made the subject of a series of anatomical investigations by Feuerborn and his co-workers. The larva has been described under a variety of names and there is no complete agreement as to the synonymy of this species. According to Rapp (1944) the following descriptions apply to this species, Dell (1905)—*Ps. sexpunctata*; Fullaway (1907)—*Ps. schizura*; Haseman (1907)—*Ps. floridica*, (1908)—*Ps. nocturnala*; Zeulzer (1909)—*Ps. sexpunctata*; Welch (1912)—*Ps. albimaculata*. Johannsen (1934), whilst admitting that his conclusions are based only on the descriptions of the larvae and pupae, and do not take the adults into consideration, would allow all but *Ps. floridica* and *Ps. albimaculata* to stand as separate species.

Individual aspects of larval anatomy have been dealt with by Haseman (1910) who gave an account of the alimentary canal and mouth parts, by Mukerji (1931) who compared the siphon with that of related Psychodid genera, and by Lloyd *et al.* (1940) who described the mandible and premandible. In addition to the foregoing, Feuerborn and his co-workers have made some substantial contributions.

Feuerborn has given an account of the larval chaetotaxy (1927*a*), the development of the head and thorax (1927*b*) and the formation of the larval tracheal and muscular systems (1927*c*). The development of the gonads and their ducts in the larva have been studied in the female by Koch (1929), and in the male by Friele (1930). The histolysis of the larval muscles and the formation of the imaginal muscles have been described by



Figs. 41–46

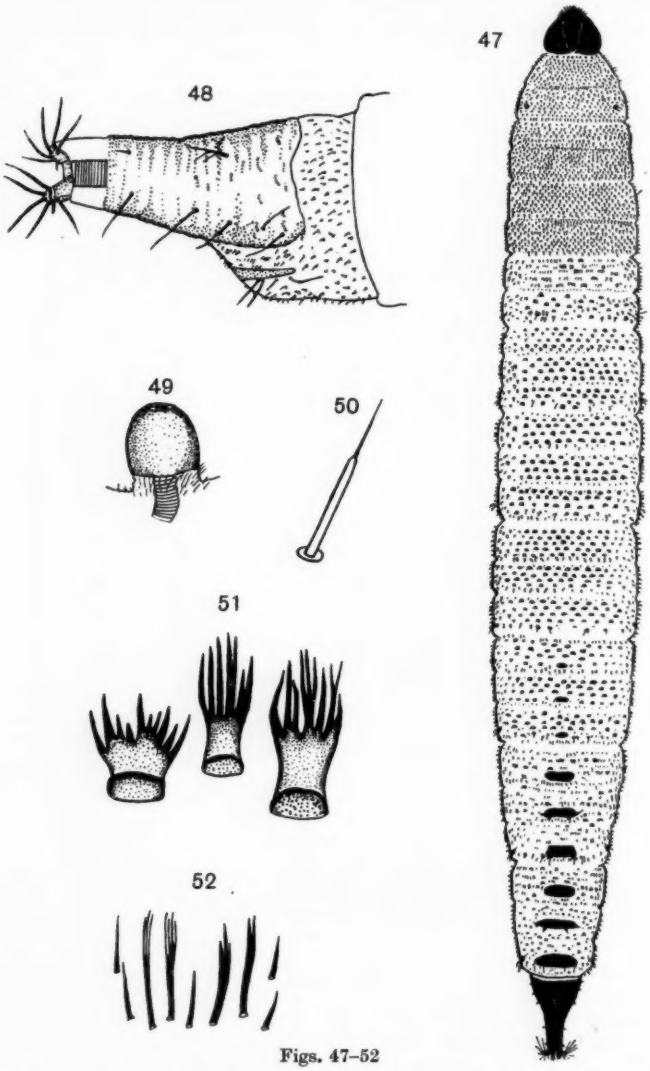
Fig. 41. Larva of *Psychoda albipennis* Zett. × 33. Fig. 42. Siphon × 69. Fig. 43. Anterior spiracle × 420. Fig. 44. Siphonal sensilla × 590. Fig. 45. Vestitural elements of dorsum of abdomen × 590. Fig. 46. Ambulatory elements × 590.

Schmidt (1928), whilst Dirkes (1928) has given an account of the development of the wing muscles. Lastly, the anatomy, histology and metamorphosis of the alimentary canal have been described by Hövener (1930).

Larva (Fig. 47), vermiform, extensible, 7·2 mm. long.

Dorsal plates present on each annulus of segments nine and ten, often vestiges of plates present on segment eight, making six or nine plates in all; plates elliptical, less than three times as broad as long; body setae of moderate length. Vestiture of fan-like combs dorsally (Fig. 51), each fringed with unequal pointed teeth; combs replaced between the segments by pointed single and double spines; ambulatory areas weak, consisting of scattered, forked hairs and combs (Fig. 52), present ventrally on the first annulus of segments four to ten, weaker on the posterior two annuli; thoracic modification seen on all three segments, with a tendency for the individual elements to coalesce and form small combs on the metathorax. Siphon (Fig. 48), five to six times as long as broad, conical, only slightly swollen at the base; ventral processes each bearing a single annular thickening; siphonal sensilla rod-

like, terminating in a hair half the length of the rod. Anterior spiracle (Fig. 49), cylindrical, domed, taller than wide.



Figs. 47–52

Fig. 47. Larva of *Psychoda alternata* Say × 23. Fig. 48. Siphon × 69. Fig. 49. Anterior spiracle × 425. Fig. 50. Siphonal sensilla × 585. Fig. 51. Vestitural elements of dorsum of abdomen × 585. Fig. 52. Ambulatory elements × 585.

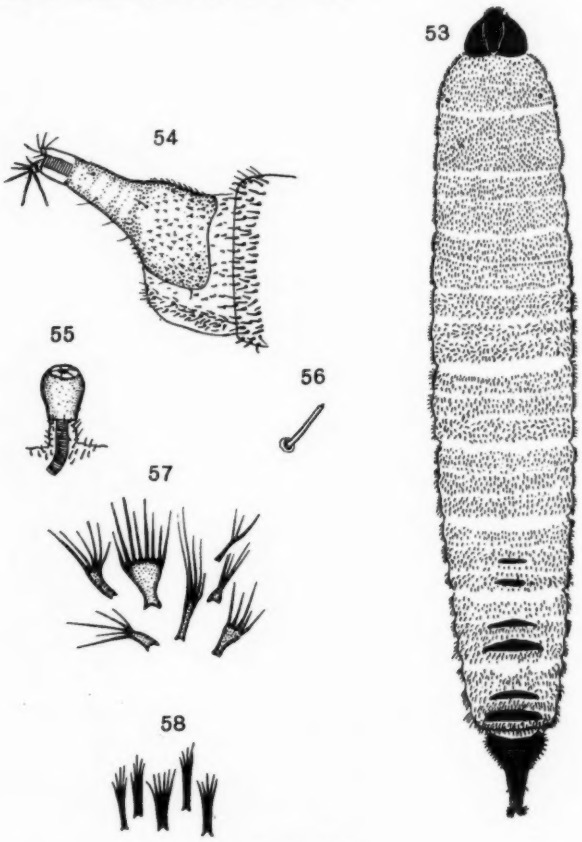
This species is the most commonly occurring sewage breeding *Psychoda*, a fact that has earned it the name of 'the trickling filter fly', and it is often present alone in beds too foul to support other species. Lundie (1940) records it breeding in sealed septic tanks. It has been found breeding in privies and urinals (Dell, 1905), in household drains, and in mud flats, (Lloyd, 1945). Saunders (1928) records it from piles of seaweed covered daily by the tide, and it has been collected from the sodden refuse of disused farm feeding troughs. It appears to show a

preference for foul and wet habitats and, like *Ps. cinerea*, has not emerged from any of the samples of field dung.

Psychoda lobata Tonn.

Larva (Fig. 53), cylindrical, rather flattened, 3·6 mm. long.

Dorsal plates present on the two posterior annuli of segments four to ten. Siphon (Fig. 54), five times as long as broad, wide basally, narrowing abruptly to the tapering distal region; basal region clothed with short, pointed spines; ventral processes bearing a single annular thickening; siphonal sensilla short, rod-like and pointed (Fig. 56). Anterior spiracle protuberant, wider apically than at the base (Fig. 55).



Figs. 53–58

Fig. 53. Larva of *Psychoda lobata* Tonn. × 33. Fig. 54. Siphon × 69. Fig. 55. Anterior spiracle × 420. Fig. 56. Siphonal sensilla × 590. Fig. 57. Vestitural elements of dorsum of abdomen × 590. Fig. 58. Ambulatory elements × 590.

of segments eight, nine and ten, making six in all; plates on segments nine and ten, strap-like, five to six times as broad as long; those on segment eight somewhat reduced; body setae inconspicuous. Vestiture consisting of a dense covering of branched hairs (Fig. 57); bands of longer hairs present behind the dorsal plates; ambulatory areas consisting of single bands of short hairs with branched tips (Fig. 58), present ventrally on the first and second annuli

The larval habitat of this species is unknown. It is a scarce species around Leeds, and it has not been recorded emerging from any of the likely breeding materials collected.

Psychoda trinodulosa Tonn.

Larva (Fig. 59), cylindrical, 3·9 mm. long.

Only two dorsal plates present, on the last two annuli of segment ten; body setae short and

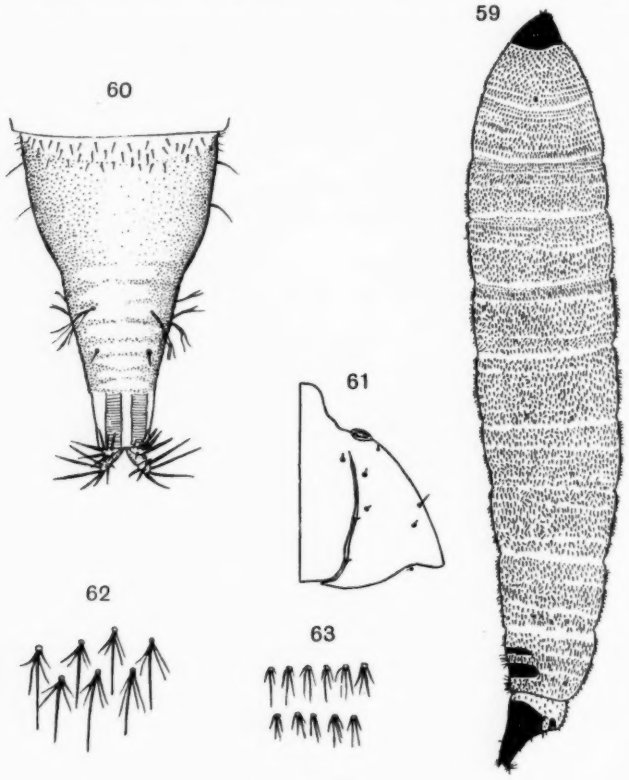
inconspicuous. Setae on head capsule short, the lateral pair not mounted on cylindrical bosses; five setae bordering epicranial suture, consisting of an anterior and a median pair, and a single posterior seta inserted just above the sharp basal bend in the suture (Fig. 61). Vestiture consisting of a shaggy coat of long hairs each bearing a group of shorter hairs around the base (Fig. 62), ambulatory areas ill-defined, consisting of patches of smaller stronger hairs bearing three or four points (Fig. 63), present on the anterior ventral margin of segments four to ten. Siphon (Fig. 60), short, conical, tapering evenly from the base to the tip; five times as long as broad; ventral processes bearing a single annular thickening; siphonal sensilla short, pointed, and rod-like. Anterior spiracle globular, slightly taller than wide.

The larva of this species has only been recorded from cow dung, in which it is quite common.

Psychoda grisescens Tonn.

Larva (Fig. 64), cylindrical, tapering, of greatest width at the metathorax, 3·3 mm. long.

Only two dorsal plates present, on the last two annuli of segment ten; body setae short and inconspicuous. Setae on head capsule short (Fig. 66); lateral pair not mounted on cylindrical bosses; four setae bordering epicranial suture, the single posterior one at the basal bend of the suture being absent. Vestiture consisting of a covering of fine short single hairs with a number of divided hairs interspersed (Fig. 67); ambulatory areas forming distinct crescents of short strong spines (Fig. 68), on the anterior margin of the second annulus of segments four to ten. Siphon (Fig. 65), six times as long as broad, consisting of a rounded base and a parallel-sided terminal portion; ventral processes bearing a single annular thickening; siphonal sensilla short,



Figs. 59–63

Fig. 59. Larva of *Psychoda trinodulosa* Tonn., lateral view × 33. Fig. 60. Siphon, dorsal view × 135. Fig. 61. Dorsal view of head capsule × 182. Fig. 62. Vestitural elements of dorsum of abdomen × 590. Fig. 63. Ambulatory elements × 590.

rod-like and pointed. Anterior spiracle globular, slightly taller than wide.

The larva of *Ps. grisescens* is commonly present in field dung and has also been recorded in a mud-flat (Lloyd, unpublished).

Psychoda setigera Tonn.

Larva (Fig. 69), cylindrical, slightly tapered at both ends, 3·3 mm. long.

Only two dorsal plates present, on the last two annuli of segment ten; body setae short and inconspicuous. Setae on dorsal region of head capsule short, lateral pair longer and mounted on cylindrical bosses (Fig. 71); five setae bordering epicranial suture, arranged as in *Ps. trinodulosa*. Vestiture consisting of a covering of fine, short, single hairs, interspersed with a number of divided hairs (Fig. 72); ambulatory areas indefinite, consisting of diffuse patches of short, strong spines on the ventral surface of the second annulus of segments four to ten (Fig. 73). Siphon (Fig. 70), seven times as long as broad, consisting of a small, swollen base and a long tubular parallel-sided distal portion; ventral processes bearing a single annular thickening; siphonal sensilla short, pointed and rod-like. Anterior spiracle globular, slightly taller than wide.

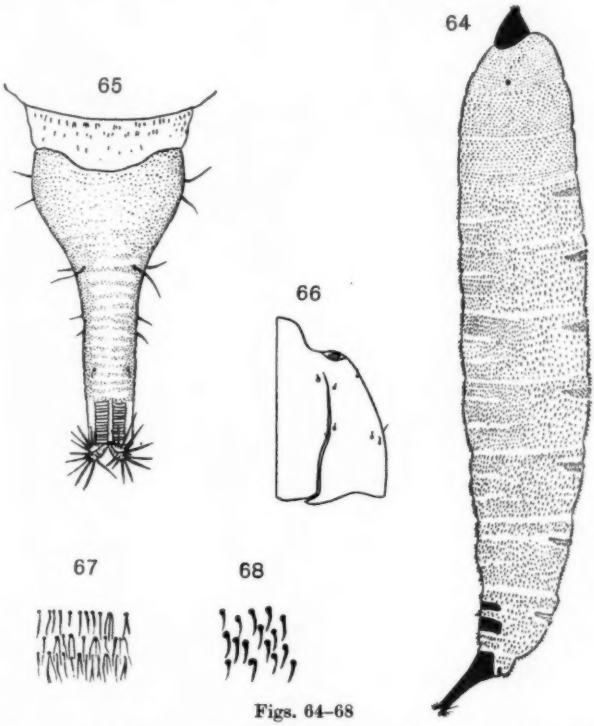
The larva of this species has been found only in cow dung, in which it is quite common.

SECTION C

Psychoda spreta Tonn.

Larva (Fig. 74), flattened, evenly tapered at both ends, 3·2 mm. long.

Dorsal plates present on both annuli of segments one to four, and on the posterior two annuli of segments five to ten, making ten pairs of plates, or twenty in all; no sign of reduction of the median plates. Body setae very long, reaching to the margin of the following segment, the lateral group in segments four to ten mounted on a small oval sclerotized plate (Fig. 75). Vestiture consisting dorsally, of a sparse covering of short, stout, single pointed spines (Fig. 78), and laterally, bordering the margins of the dorsal plates, of longer and stouter



Figs. 64–68

Fig. 64. Larva of *Psychoda grisescens* Tonn., lateral view × 33. Fig. 65. Siphon, dorsal view × 135. Fig. 66. Dorsal view of head capsule × 182. Fig. 67. Vestitural elements of dorsum of abdomen × 590. Fig. 68. Ambulatory elements × 590.

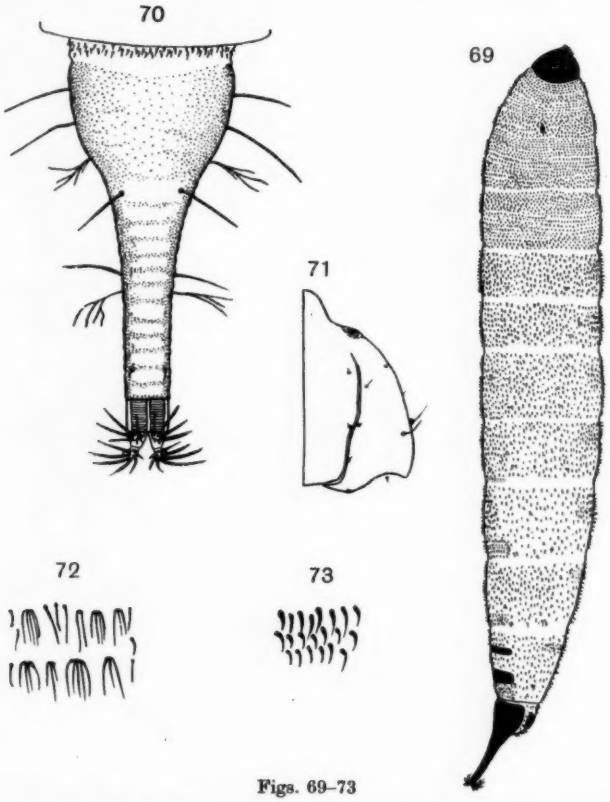
hairs (Fig. 80); ambulatory areas consisting of branched comb-like hairs (Fig. 79), forming an almost complete ring on the first annulus of segments four to ten, reaching nearly to the plate of the second annulus, and being feebly developed on the third; thorax evenly covered with small close-set spines. Siphon (Fig. 75), six to seven times as long as broad, consisting of a swollen base and tapering terminal portion; ventral processes each bearing two annular thickenings; siphonal sensilla (Fig. 76), straight and bluntly pointed. Anterior spiracle cylindrical, domed, taller than wide (Fig. 77).

This species has not emerged from any of the samples of likely breeding materials collected in the field, but jars of rotting grass-cuttings and scalded *Phormidium*, which were exposed in woodland for a week, both subsequently produced adults. As *Ps. spreta* has not emerged from any of the dung samples it seems probable that this species normally inhabits rotting vegetation.

Psychoda brevicornis Tonn.

Feuerborn (1913) gives a brief description of an unidentified *Psychoda* larva he found in dung which was characterized by its small size and by the peculiar arrangement and shape of the hairs and spines on the dorsal and lateral surfaces. As he gives no figure it is impossible to be certain as to the identity of the larva, but the description would apply very well to that of *Ps. brevicornis* Tonn., a species that had not, at that time, been described. Apart from this rather uncertain record, there has been no description of the larva of this species.

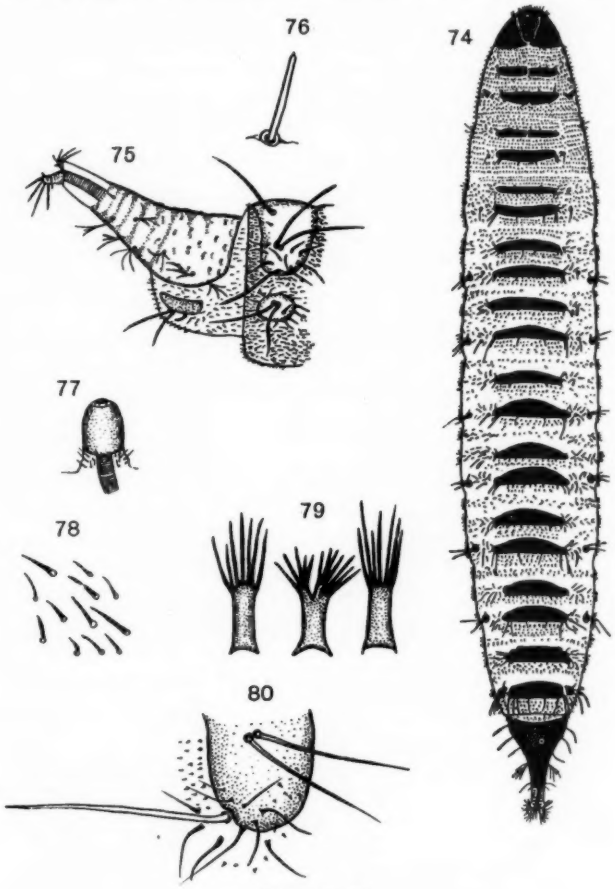
Larva (Fig. 81), much flattened, 2·8 mm. long, with a prominently developed lateral fringe of hairs.



Figs. 69–73

Fig. 69. Larva of *Psychoda setigera* Tonn., lateral view × 33. Fig. 70. Siphon, dorsal view × 135. Fig. 71. Dorsal view of head capsule × 182. Fig. 72. Vestitural elements of dorsum of abdomen × 590. Fig. 73. Ambulatory elements × 590.

Dorsal plates present on each annulus of segments one to four and on the two posterior annuli of segments five to ten, making ten pairs, or twenty in all. Plates bearing a variety of hairs and spines as follows: posterior dorsal plate of segments two to ten bearing three hyaline, palmately branched setae on the lateral margin (Fig. 83), posterior plate a lateral fringe of four plumose setae. Terminal segment (Fig. 84), flattened, sclerotized dorsally, semicircular in outline and not tubular as in other forms; bearing a minute, transparent, conical siphon at the posterior end; dorsal and ventral processes of siphon very much reduced; siphonal sensilla rod-like terminating in a hair, borne, not on



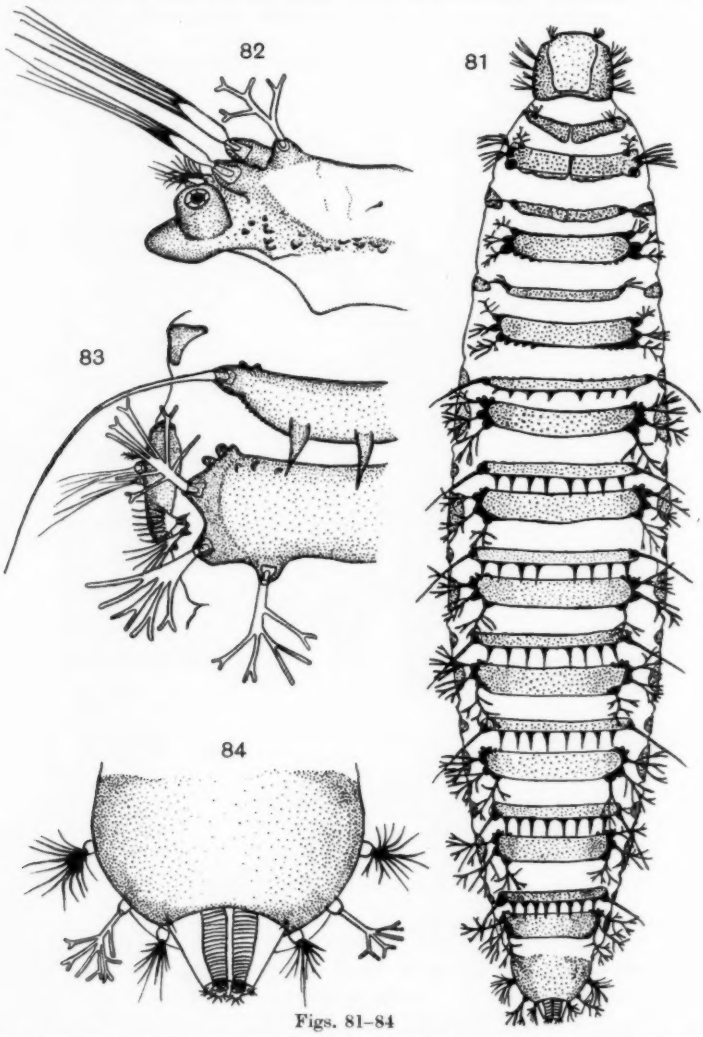
Figs. 74–80

Fig. 74. Larva of *Psychoda spreta* Tonn. × 33. Fig. 75. Siphon × 69. Fig. 76. Siphonal sensilla × 590. Fig. 77. Anterior spiracle × 420. Fig. 78. Vestitural elements of dorsum of abdomen × 590. Fig. 79. Ambulatory elements × 590. Fig. 80. Vestitural elements bordering lateral margins of dorsal plates × 150.

of first segment bearing two long brush-like setae near to the anterior spiracle, and one palmate seta (Fig. 82); anterior dorsal plate of segments four to ten bearing a single, long, bow-like lateral seta, and a posterior fringe of seven or eight stout, sclerotized thorns. Two lateral plates present on segments four to ten, the larger posterior one bearing two brush-like and one pennate setae. Head quadrate, bearing the siphon, but close to the base. Terminal segment fringed with two brush-like and one palmate setae. Vestiture consisting of a covering of fine, single pointed hairs between the dorsal plates and on the ventral surface. Anterior spiracle (Fig. 82), large, cylindrical, directed postero-dorsally, situated on the hind margin of the second prothoracic plate.

The larva of this species differs considerably from those of the other species described in this paper both in its appearance and in its behaviour. Instead of burrowing into the dung with only the tip of its respiratory siphon exposed, as is the usual living habit also helps to explain why this species suffices with a very short respiratory siphon, when other dung-living species have relatively long ones.

It is a pleasure to acknowledge my indebtedness to Dr Ll. Lloyd for his help and advice, and to Professor E. A. Spaul for discussion in the course of this work. I am also indebted to the Department of Scientific and Industrial Research for a grant which has permitted this study to be made.



Figs. 81–84

Fig. 81. Larva of *Psychoda brevicornis* Tonn., dorsal view × 50. (The ventro-lateral fringe of hairs has been omitted.) Fig. 82. The posterior prothoracic plate, bearing the anterior spiracle × 175. Fig. 83. One of the abdominal segments, dorsal view × 175. Fig. 84. The terminal segment, dorsal view × 175.

REFERENCES

DELL, J. A. (1905). *Trans. R. Ent. Soc. Lond.* p. 293.

DIRKES, L. (1928). *Z. Morph. Ökol. Tiere*, **11**, 182.

FEUERBORN, H. J. (1913). *S.B. naturh. Ver. preuss. Rheinl. Westf.* B, 11.

FEUERBORN, H. J. (1927*a*). *Zool. Anz.* **70**, 167.

FEUERBORN, H. J. (1927*b*). *Zool. Anz.* **70**, 315.

FEUERBORN, H. J. (1927*c*). *Zool. Anz.* **71**, 249.

FEUERBORN, H. J. (1933). *Arch. Hydrobiol. Plankt.*, Suppl. **11**, 55.

FRIELE, A. (1930). *Z. Morph. Ökol. Tiere*, **18**, 249.

FULLAWAY, D..T. (1907). *Ent. News*, **18**, 386.

HASEMAN, L. (1907). *Trans. Amer. Ent. Soc.* **33**, 299.

HASEMAN, L. (1908). *Ent. News*, **19**, 274.

HASEMAN, L. (1910). *Ann. Ent. Soc. Amer.* **3**, 277.

HÖVENER, M. (1930). *Z. Morph. Ökol. Tiere*, **18**, 74.

JOHANNSEN, O. A. (1934). *Mem. Cornell Agric. Exp. Sta.* **164**, 1.

JOHNSON, J. W. H. (1914). *J. Econ. Biol.* **9**, 127.

KEILIN, D. & TATE, P. (1937). *Parasitology*, **29**, 247.

KLOET, G. S. & HINCKS, W. D. (1945). *A Check-list of British Insects*. Stockport.

KOCH, M. (1929). *Z. Morph. Ökol. Tiere*, **14**, 1.

LLOYD, LL. (1945). *J. Inst. S.P.* p. 3.

LLOYD, LL., GRAHAM, J. F. & REYNOLDSON, T. B. (1940). *Ann. Appl. Biol.* **27**, 122.

LUNDIE, M. (1940). *J. Inst. S.P.* p. 212.

MALLOCH, J. R. (1917). *Bull. Ill. Lab. Nat. Hist.* **12**, 161.

MUKERJI, S. (1931). *Indian J. Med. Res.* **19**, 433.

MUTTKOWSKI, R. A. (1915). *Bull. Wis. Nat. Hist. Soc* **13**, 109.

RAPP, W. F. (1944). *J. N.Y. Ent. Soc.* **52**, 201.

SAUNDERS, L. G. (1928). *Entomologist.* **61**, 209.

SCHMIDT, E. (1928). *Z. Morph. Ökol. Tiere*, **13**, 117.

WELCH, P. S. (1912). *Ann. Ent. Soc. Amer.* **5**, 411.

ZAVATTARI, E. (1924). *Redia*, **15**, 191.

ZEULZER, M. (1909). *Mitt. Pruf. Anst. Wasservers. Berl.* **12**, 213.

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THE STRUCTURE OF THE CUTICLE IN *EOMENACANTHUS STRAMINEUS* (NITZSCH), (MALLOPHAGA)

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(With Plate I)

In recent work on the deposition of the epicuticle, Wigglesworth (1946) has shown that, in *Rhodnius prolixus*, not only is the epicuticle a composite structure comprising several distinct layers, but that the pore canals, which hitherto have been believed to terminate at the base of the epicuticle, do in fact penetrate its innermost regions. Wigglesworth extends the term epicuticle to cover the four outer layers of the cuticle in *Rhodnius*. The innermost of the four is a cuticulin layer which is followed by a polyphenol layer, a wax layer and an outer cement layer respectively. The pore canals penetrate the cuticulin layer.

This paper deals with another case in which the existence of separate layers in the epicuticle penetrated by pore canals can be demonstrated. A full account of the method used to prepare the sections of cuticle has been described elsewhere (Webb, 1946).

In *Eomenacanthus stramineus* the cuticle of the postero-ventral region of the head and of the lateral regions of each sternum of the abdomen is abnormally thick. This increase in thickness is due entirely to an expansion of the epicuticle and the exocuticle and appears to have taken place in a lateral as well as a vertical direction. It is possible, therefore, in this expanded region, to see details of epicuticular structure which are obscure in normal cuticle chiefly owing to their smallness of size. The normal cuticle of *Eomenacanthus* is about 3μ thick and of this about 2μ is endocuticle, the remainder being exocuticle and epicuticle. In the expanded regions, the endocuticle remains 2μ thick but the exocuticle and epicuticle together may be up to 10μ or 12μ thick, the epicuticle itself being from 2 to 4μ thick. Sections of cuticle were cut 5μ thick and stained with Ehrlich's haematoxylin and orange 'G'. The endocuticle stained strongly with orange 'G' and showed a horizontal lamination in its structure. The exocuticle remained unstained and showed no apparent structure. Following this a composite layer comprising at least three regions could be seen. The thin innermost layer, lightly stained with haematoxylin, was followed by a central unstained region and an outer broader layer stained strongly with haematoxylin. Pore canals arising from the hypodermal cells and traversing the endocuticle and the exocuticle were also seen to pass through these outer layers giving them an appearance of discontinuity. In the sections from the ventral region of the head there is a discrete layer situated above the cuticle. This is evidently the 'cement' layer described by Wigglesworth in *Rhodnius* cuticle. It presumably formed a protective covering over the wax layer and was freed by the removal of this wax during the process of embedding. Sections of cuticle from the abdominal sterna, however, show no trace of this layer, but, as it is not readily distinguishable until it has separated from the remainder of the cuticle, there is no proof of its absence in this region. Photomicrographs of typical sections of cuticle both from the head and from an abdominal sternum are shown in Plate I, figs. 1–3. In fig. 1, A, the normal cuticle from the region of the head is seen to merge into the expanded cuticle. In the expanded region, pore canals traverse the cuticle as far as the 'cement' layer which, towards the left-hand side of the figure, has slightly separated from the remainder of the cuticle. Fig. 2, B, shows a similar section of cuticle from the head with the 'cement' layer ruptured and distinctly raised. Fig. 3, C, shows a section through the cuticle of an abdominal sternum in which the merging of normal cuticle into expanded cuticle is clearly seen and the relative thickness of epicuticle, exocuticle and endocuticle in each region can be gauged. In the expanded region of the cuticle in this figure the presence of three distinct layers comprising what is presumably the epicuticle is well marked.

It is not clear how far the structure of normal cuticle can be deduced from that of an abnormal region, nor how far the various layers seen in the expanded cuticle of *Eomenacanthus* may be homologized with those described by Wigglesworth in *Rhodnius*. It is evident from the abnormal staining properties of the epicuticle and exocuticle in *Eomenacanthus* that differences other than thickness may be involved. The outer, detached 'cement' layer is doubtless similar to that found in *Rhodnius* and the underlying wax layer may be considered to have been lost. This leaves three layers to be accounted for of which two seem to be present in *Rhodnius*. If the outer layer of these three which

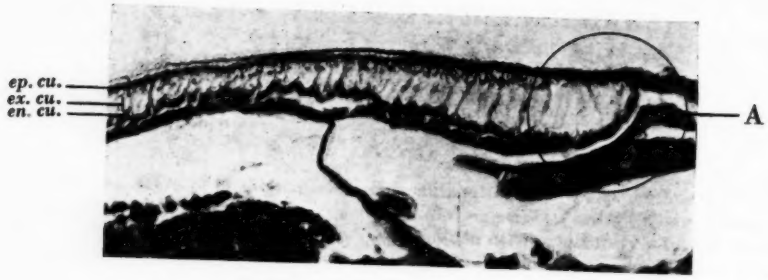


Fig. 1

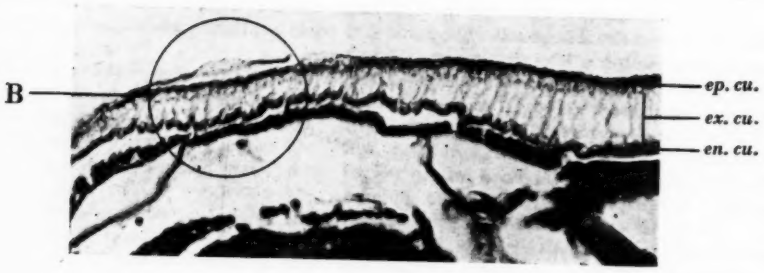


Fig. 2

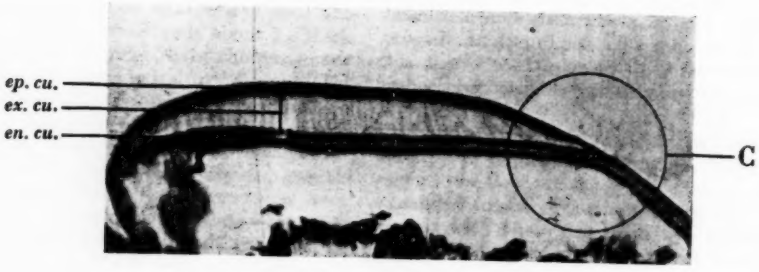
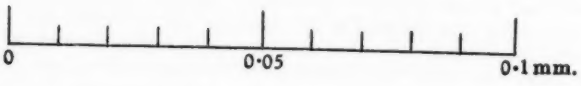


Fig. 3



stains strongly with haematoxylin is homologous with the polyphenol layer in *Rhodnius* and the centre non-staining layer with the cuticulin layer in that insect, then the thin, innermost layer staining lightly with haematoxylin is either a new structure not represented in *Rhodnius* cuticle or one which, owing to its extreme thinness, has so far been overlooked.

I wish to express my thanks to Dr V. B. Wigglesworth for his helpful criticism and for permission to quote from his unpublished work.

Index to lettering on Plate I, figures 1 to 3

A. Normal cuticle merging into expanded cuticle in the head region. B. 'Cement' layer ruptured and separated from the remainder of the cuticle. C. Normal cuticle merging into expanded cuticle in an abdominal sternum. *en. cu.* endocuticle; *ep. cu.* epicuticle; *ex. cu.* exocuticle.

REFERENCES

WEBB, J. E. (1946). *Proc. Zool. Soc. Lond.* **116**, 49–119.

WIGGLESWORTH, V. B. (1946). *Proc. roy. Soc.* B (in the Press).

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THE SEASONAL ACTIVITY OF THE TICK, *IXODES RICINUS* L., IN WALES

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(With 12 Figures in the Text)

CONTENTS

	PAGE
1. Introduction	72
2. Tick activity on grassland	72
(i) Technique	72
(ii) Seasonal activity of nymphs	73
(iii) Seasonal activity of males and females	75
(iv) Seasonal activity of larvae	77
3. Tick activity on farm stock	77
(i) Technique	77
(ii) Seasonal prevalence of the tick on cattle	79
(iii) Seasonal activity of the tick in other parts of Wales	79
(iv) Engorgement period of female ticks on cattle	82
(v) Comparative susceptibility of cattle breeds to tick infestation	82
(vi) Tick infestation and reproductive condition of the host	83
(vii) Seasonal prevalence of the tick on sheep	84
(viii) Seasonal prevalence of the tick on horses	84
4. Summary	85
References	85

1. INTRODUCTION

The present studies were undertaken to elucidate certain aspects of the ecology of the sheep tick, *Ixodes ricinus* L., in Wales as a prerequisite to control measures by grassland improvement and treatment of cattle. Extensive and detailed information on the seasonal incidence of this tick in the north of England and in Scotland has been given by MacLeod (1939) and Milne (1945*a*), with special reference to its occurrence on sheep as the principal host. A summary of MacLeod's findings shows that in Argyllshire, Perthshire, Selkirk and Northumberland, tick incidence is greatest in spring and decreases markedly in early summer, with a slight recrudescence of activity in autumn, and is almost completely absent in winter. It attains its maximum activity in early May in Argyllshire, in the middle of May in Perthshire and towards the end of May in the Borders and Northumberland. Milne (1945*a*) has shown there are two peaks of activity, one in spring and the other in autumn at Crag, in south-western Cumberland, but only one in spring at Hethpool, in north-eastern Northumberland. In the Hill o' Fare, in Aberdeenshire, there is a single peak of summer activity (Hendrick, Moore & Morison, 1938). Milne (1945*a*) has discussed the theories of Wheler (1899), Falke (1931), Totze (1933) and MacLeod (1939) which have been put forward to explain the seasonal activity of the ticks, and while he believes all may contribute something towards the truth, he is of the opinion that the temperature-cum-humidity of the micro-climate deserves further investigation.

The present paper embodies the results of investigations undertaken on both hill and lowland grazings in Wales. Hitherto, the work on tick ecology in the north of England and Scotland has been related primarily to sheep as the principal host (Milne, 1945*c*). In mid-Wales, at least in the north of Cardiganshire, sheep are likewise liable to heavy infestations and according to Rowlands (1945) the tick in North Wales is mainly related to staphylococcal pyaemia in sheep, which reaches a very serious proportion on certain sheep walks. On the contrary, cattle appear to be the preferential host in South Wales and much of the data included in the present paper relate to this aspect.

2. TICK ACTIVITY ON GRASSLAND

(i) *Technique.* The method adopted for the recovery of nymphs from grassland is based on the dragging of a white, woollen, single-bed sized blanket over the suspected tick-infested pastures. A similar method has been employed qualitatively by MacLeod (1932) and others, and quantitatively by Milne (1943). The unit drag length employed in the present work, in common with that used by the latter worker, was 50 yards. At the end of the drag all stages of the tick were removed and the populations of the different phases recorded.

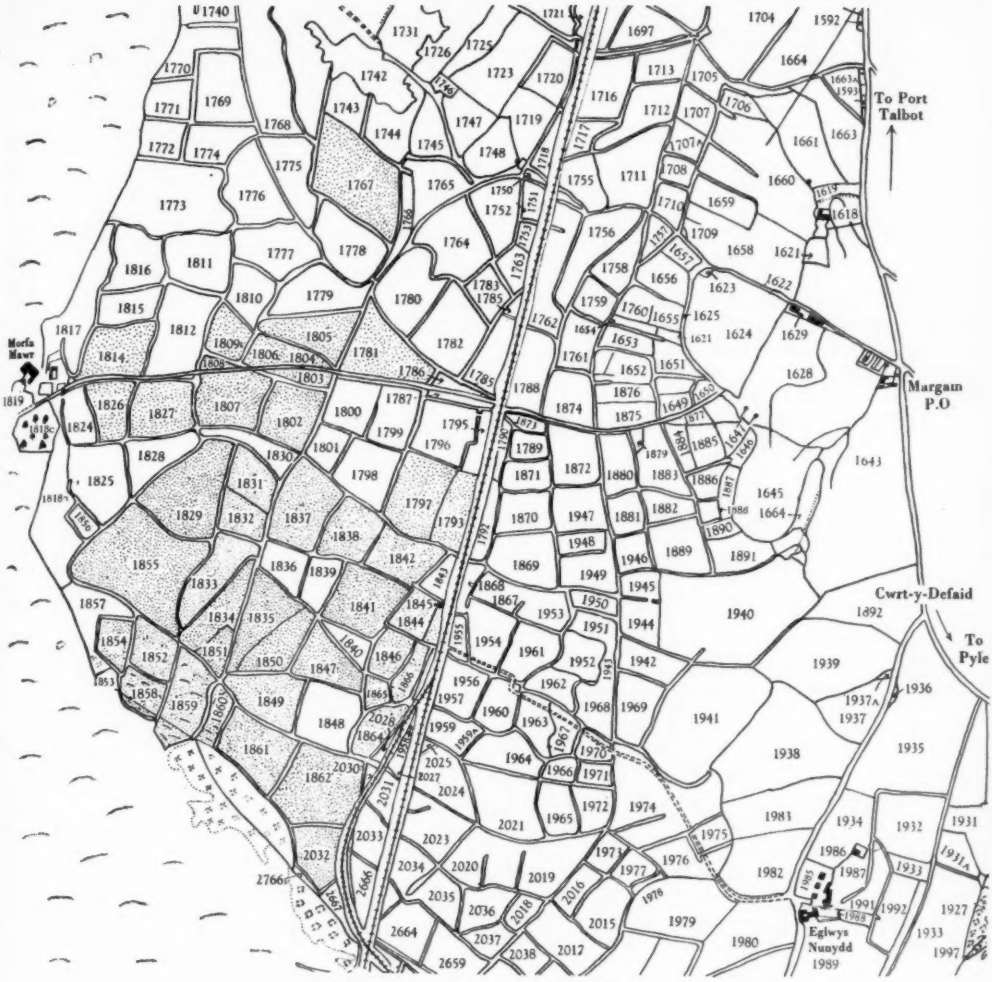


Fig. 1. Tick-infested fields on Margam Moors, Glamorgan (1944–5).

(ii) *Seasonal activity of nymphs.* The results outlined in the present paper are based on data assembled during 1944 and 1945, thus:

Date	Average no. of days of dragging per week	No. of plots dragged on each occasion	Average no. of drags per week*
8. iii–31. x. 44	3	5	23
20. iii–31. x. 45	3	8	96

* The figures do not include the drags made on other fields or tracts of land to determine the presence or absence of ticks.

In 1944, the fields were visited, on an average of three separate days each week between 21 April and 31 October, but prior to this period six blanket drags had been carried out commencing on 8 March. During this period the arithmetical mean of the combined drags was 5·7, with a standard deviation of 1·29. Towards the end of April, the population in all pastures under investigation increased rapidly attaining a peak during the first to the third week of May, when the mean per drag was 35·6 with a maximum of 78·0 at the beginning of the third week (Fig. 2). In field O.S. Margam (1941), 1808 (Fig. 1) the following

data were obtained within the 7-day period 15–22 May.

Date	Drag no.	Recovered ticks ♂	♀	o	L.
15 May	1	1	0	20	0
	2	0	0	47	0
16 May	1	2	0	78	0
	2	1	0	41	0
20 May	1	1	0	19	0
	2	0	1	9	0
	Total	5	1	214	—
	Mean	0·53	0·13	35·6	—

From 23 May to the third week in July the nymphs decreased gradually in numbers and became non-recoverable by the blanket method in August. Despite intensive dragging of stretches of land known to have harboured the tick earlier in the season and of virgin areas in their immediate vicinity, no further nymphs were recovered during the remainder of the year, with the exception of one nymph on 31 August (Fig. 2).

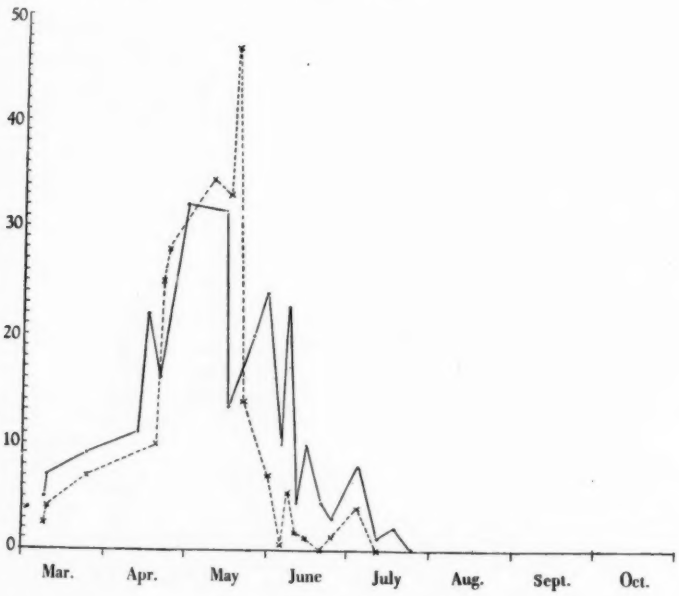


Fig. 2. Nymphal tick activity curves in Glamorgan, Margam Moors (1944).

In 1945, 30 acres of moor (O.S. Margam (1941), 1829 and 1855) known to be tick infested, as shown by the 1944 investigation, were subdivided into eight contiguous plots (*A–H*), with individual areas of some 3·75 acres and arranged as in Fig. 3. The plots were regarded as separate entities and simultaneous quadruple draggings for the different stages of the tick were undertaken on each plot. The lack of uniformity of vegetation cover was the principal difficulty encountered in employing the blanket method on the moors, a factor which, as already indicated by Milne (1943), influences its efficiency. In view of this uneven vegetation, areas as far as possible of uniform cover were examined for the determination of the seasonal activity curve. The experimental area had been grazed by cattle up to 1942, when, due to heavy losses from Redwater Fever, the practice was discontinued. Subsequently it was grazed by horses and sheep. In 1945, the latter were not introduced into the area until June and were again removed at the end of August.

This part of the moor is characterized topographically by longitudinal depressions which run the entire length of the plots at intervals of 12–16 yards. The alternating ridges are somewhat higher, better drained and grazed harder by stock. The vegetation is greatly influenced by these surface features. In the hollows, the drainage is much impeded and the dominant plants consist of *Molinia* spp. and *Juncus* sp. On the ridges, where there is much 'run-off' of surface water, *Agrostis tenuis* Sibth, predominates, often forming as much as 70% of the herbage. Besides these plants, 'flags', *Iris pseudacorus* L., are prevalent, particularly in the vicinity of the ill-drained ditches. In

general, it may be stated that rush, *Juncus communis* L., characterizes some 70% of the area in plots *B*, *C* and *D*, and about 50% in *E*, *F*, *G* and *H*, while in *A* the herbage consists mainly of rough grasses which, to a large measure, are grazed hard.

It will be seen from Figs. 4, 5, 6, 7 that the nymphal activity in 1945 followed the same seasonal trend as in 1944. With a view to determine the degree of variation on the different plots, the figures at the maximum period of activity (3–10 May, in 1945) are statistically treated. This peak period is considered the best phase for such an analysis as the tick population at this stage has attained equilibrium with its environment, at least the majority of the nymphs that become active at any one time in the spring season are then most readily recovered (Table 1).

	Sum of squares	D.F.	Variance
Total	11554·4	95	—
Days	724·7	2	362·0
Plots	1228·2	7	175·46
Error	9601·5	86	101·2

For the period under review there is a significant difference between the daily population totals over the eight plots. While differences in tick intensity are apparent over the 30 acres involved, the peak of activity is attained within the same 8-day period. In plots *D* and *E*, the recorded peak is on 3 May, in plots *A*, *B*, *F*, *G* and *H* on 8 May and in plot *C* on 10 May. In this connexion it is noteworthy that as Milne (1945*a*) points out the true peak may be anywhere within plus or minus half an interval of the recorded peak. Relative to the 1944 figures, the maximum number of ticks at the mode was somewhat lower in 1945 and the peak of activity occurred about a week later, but allowing for a slight elasticity of dates there is a close correspondence in the general contour of the graph in both years. No recrudescence of nymphal activity in autumn of either season has been apparent. A variation between the nymphal populations on the different plots is also apparent and their significant levels can be determined by the application of the *Z* test, thus:

S.E. of the mean percentage for each plot = 101·2/4.

S.E. of the difference between two such means = 101·2 × 2/4.

As the number of degrees of freedom is over 30, i.e. 86, a significant difference can be considered as being twice the standard error = 7·113 × 2 = 14·226. The plots can thus be classified into four numerical groups according to their mean nymphal populations over the period in question and they are statistically significant. From this data it is apparent that plot *E* has a significantly greater population than plots *G*, *B* or *A*, and that plot *C* is greater than *A*, whereas plots *C*, *D*, *F*, *H*, *B* and *G* lie very much on the same level of significance.

(iii) *Seasonal activity of males and females.* From the data assembled in 1944 and 1945 it is evident

Table 1. *The nymphal tick populations from plots* (*A*–*H*) *on Margam Moors* (3–10. v. 45)

Plot index	3 May (*A* 1)				8 May (*B* 1)				10 May (*C* 1)			
Drag nos. ...	1	2	3	4	1	2	3	4	1	2	3	4
A	27	2	10	12	19	23	12	5	0	3	1	2
B	31	14	12	2	23	12	25	17	18	2	0	2
C	13	9	24	10	17	27	13	19	35	35	19	17
D	48	45	11	9	2	19	0	24	23	12	38	0
E	29	35	22	27	27	30	20	31	9	12	23	35
F	34	27	15	3	13	23	17	27	0	7	24	32
G	12	4	14	6	17	31	10	18	0	1	25	23
H	21	41	1	0	45	23	15	19	10	1	12	8
Total	215	177	109	69	163	188	112	160	95	73	142	119

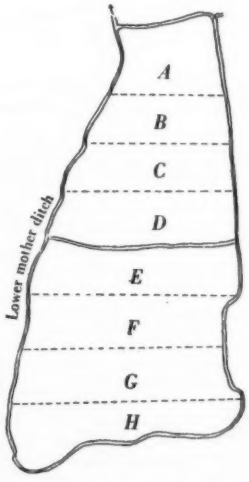
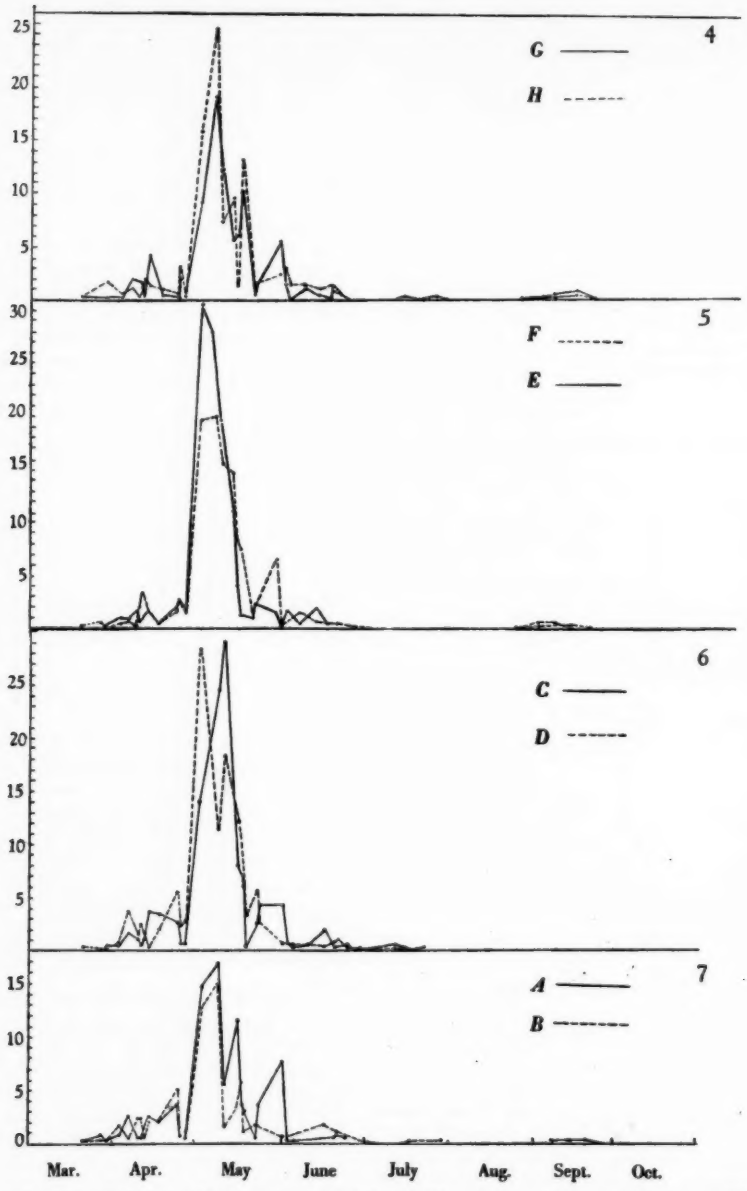


Fig. 3. Arrangement of plots on Margam Moors (see text and Figs. 4–7).

From columns *A* 1, *B* 1 and *C* 1, the analysis of variance may be calculated as follows, where the correction factor is 28016·6.



Figs. 4–7. Nymphal tick activity curves on plots (*A–H*) on Margam Moors, Glamorgan (1945).

that the recovery of adult males and females is decidedly lower than that of the nymphs by the blanket method, for which an explanation is offered by Milne (1943). Despite this fact, the seasonal activity curves of males, females and nymphs are similar and concurrent, the peaks occurring at approximately the same dates. Further, that the sex ratio, when based on the number of males and females recovered from grassland by the blanket method, is subject to variation

Table 2. Ixodes ricinus *L.*
Male : Female sex ratios, 1944–5

	1944			1945	
	♂	♀		♂	♀
March	1·0	1·63	March	1·0	0·81
April	1·0	1·33	April	1·0	1·06
May	1·0	1·30	May	1·0	1·26
June	1·0	1·80	June	1·0	1·20
July	1·0	3·0	July	1·0	2·76
August	1·0	4·0	August	1·0	1·03

follow a Poisson distribution. Due to the irregularity of the 'drop-off' of engorged females and subsequent points of oviposition, together with variation in suitability of environment, marked patchiness results in the sample counts of larvae, being almost always more variable than this law predicts. In view of the impracticability of counting individual larvae and its unreliability as a population index, only the number of larval clusters per blanket drag were considered in the present investigation. A study of the data presented in

Table 3. *Larval clusters and female ticks obtained by blanketing in* 1944–5

Date (1944)	Larval clusters per blanket	Date (1945)	Larval clusters per blanket	Females per blanket
8 April	0·054	20 March	0·031	0·031
17 April	0·074	27 March	0·031	0·281
24 April	0·000	6 April	0·031	0·218
1 May	0·036	11 April	0·000	0·156
8 May	0·153	17 April	0·000	0·375
15 May	0·185	24 April	0·031	0·972
22 May	0·089	25 April	0·031	0·594
29 May	0·197	27 April	0·000	0·281
5 June	0·180	3 May	0·000	0·437
12 June	0·050	11 May	0·000	0·219
19 June	0·000	15 May	0·094	0·187
26 June	0·197	17 May	0·188	0·465
2 July	0·094	22 May	0·063	0·437
9 July	0·000	12 June	0·000	0·156
16 July	0·188	19 June	0·281	0·031
23 July	0·272	26 June	0·188	0·015
30 July	0·000	2 July	0·031	0·007
6 August	0·000	7 July	0·062	0·000
13 August	0·000	16 July	0·000	0·000

from year to year and that in general the latter are in a preponderance (Table 2). This does not appear to be the case in all instances, however, as Milne (1945*a*) found in the north of England that males were more abundant than females on the 24 and 31 May 1943. Under laboratory conditions of breeding, MacLeod (1932) discovered, on the other hand, that the sex ratio of female:male was 1·80:1·0, a relationship only attained in June in the 1944 field studies in South Wales.

Although the peak of activity curves in both nymphs and adults are essentially coincident, the latter (i.e. males and females) remain active for a longer period than the nymphs.

(iv) *Seasonal activity of larvae.* Normally, the larvae are found in clusters on the blanket, each cluster developing from the egg mass of a single female, from which focus movement in a horizontal direction is minimal (Arthur, 1945). If individual larvae occurred at random and entirely independently of each other, that is, if there was no tendency even for very localized patchiness, the numbers found in different sampling units would

Table 3 shows that the larval-cluster peak in 1945 was at least three weeks later than that of females. Contrary to the findings of Milne (1945*a*) in the north of England, larvae were encountered in 1945 in South Wales as early as 20 March, being recovered by dragging, and their occurrence coincided with the first appearance of females. They were subsequently obtained on 27 March and 6 April.

3. TICK ACTIVITY ON FARM STOCK

The sheep tick has a wide range of mammalian and avian hosts (MacLeod, 1932, and others) and the term 'farm stock' adopted in the present paper refers to cattle, sheep and horses.

(i) *Technique*

(*a*) *Tick counts on sheep.* The method adopted for counting ticks on sheep in the present studies was similar to that described by Milne (1943).

(*b*) *Tick counts on cattle.* As in the case of sheep counts, only attached females were considered and

in order to ascertain the most suitable area for population estimates on cattle, counts on the fore- and hindquarters of thirty head of stock were undertaken at 7–11 day-intervals. The forepart was considered as being essentially similar to that examined in sheep and the hind region as the entire hindquarters including the groin and udder. Preliminary observations were commenced in 1944, and further supplemented by a larger number of counts in 1945. This information is tabulated below (Table 4).

Table 4. *Comparison of female tick populations on the fore- and hindquarters of dairy cows*

	Average no. of ticks/host on hindquarters (H), forequarters (F)		Differences in mean populations H–F		Square of differences $(H-F)^2$
Host index	H	F	+	−	
1	12·5	13·6	—	1·1	1·21
2	7·6	7·6	—	—	—
3	6·3	6·3	—	—	—
4	6·1	5·4	0·7	—	0·49
5	1·5	2·2	—	0·7	0·49
6	23·5	21·6	1·9	—	3·61
7	26·0	22·1	3·9	—	15·21
8	13·2	13·5	—	0·3	0·09
9	16·5	16·3	0·2	—	0·04
10	3·6	3·0	0·6	—	0·36
11	9·4	7·9	1·5	—	2·25
12	10·8	8·3	2·5	—	6·25
13	23·0	12·5	10·5	—	110·25
14	27·1	15·2	11·9	—	141·61
15	13·5	9·5	4·0	—	16·0
16	16·4	4·8	11·6	—	134·56
17	13·6	12·2	1·4	—	1·96
18	7·6	9·8	—	2·2	4·84
19	5·9	7·0	—	1·1	1·21
20	3·0	3·9	—	0·9	0·81
21	3·5	2·5	1·0	—	1·0
22	22·1	17·1	4·0	—	16·0
23	17·6	15·0	2·6	—	6·76
24	78·2	43·1	35·1	—	1232·01
25	103·2	92·0	11·2	—	115·44
26	12·4	9·8	2·6	—	6·76
27	12·0	5·7	6·3	—	39·69
28	15·8	12·3	3·5	—	12·25
29	1·4	2·1	—	0·7	0·49
30	1·8	3·0	—	1·2	1·44
			+117·0	−8·2	1873·08

Then:

Sum of the differences squared

$$= 1873{\cdot}08 - \frac{(108{\cdot}8)^2}{30} = 1478{\cdot}50.$$

Mean difference in ticks = 3·63.

S.E. of mean difference $= \frac{1478{\cdot}5}{29 \times 30} = 1{\cdot}31.$

$$t = \frac{3{\cdot}63}{1{\cdot}31} = 2{\cdot}7709.$$

Since the value of $t = 2{\cdot}7709$, reference to the table shows that for 29 degrees of freedom, the probability of exceeding this value purely by chance is less than 0·01, indicating that the mean difference in favour of ticks on the hindquarters is definitely significant. Preference for this region is probably due to the larger area of hairless skin which in turn is more easily penetrated by the mouth parts of the tick (Arthur, 1946). Accordingly, the hindquarters were selected as the standard region for sample counts on cattle in the present studies. In 1944, the writers noted the presence of ticks on three horses grazing infested pastures on Margam Moors (O.S. Margam (1941) 1829 and 1855). They were young animals (two 2-year old and one 1-year old) that had continually grazed the moors since October 1943 and thus experienced tick activity throughout the season. Difficulty was encountered in making accurate counts of the gorged females on them, as the horses had not been 'broken in', thus rendering their handling well-nigh impossible. By arousing their instinct of curiosity by enticing them towards

the blanket it was found possible, however, for the operator to make approximate estimates of the numbers present on the hind region, as indicated for cattle. The results obtained must be treated with reserve and no statistical analysis of the data has been attempted.

(ii) *Seasonal prevalence of the tick on cattle.* The determination of tick incidence in the Margam district was made on five farms in the scrub-pasture zone, each farm being visited at 7–11 day-intervals over a period of 8 months, from May to December in 1944. On each farm counts were made on a representative sample of the dairy stock, being on eight cattle at Longlands Farm, fifteen at Graig-Goch Farm, sixteen at Nant-y-Neuadd Farm, eight at Troedyrhiw Farm and five at Rhydybontpren Farm.

The population at each centre showed a rapid increase in the first week of May (Fig. 8), attaining a maximum peak on 14 May, with a mean population of 17 ticks, the maximum and minimum being 27 and 12, respectively. Thereafter, the populations at all centres decreased gradually to the third week of July and then remained at a low level until late August, rarely during this period exceeding 2 ticks per beast. Following this period of inactivation, a secondary peak occurred with a maximum of 10·8 ticks per beast on 11 September. From this date there was a gradual decline in numbers of the tick to 29 November, after which period none was found until 27 March 1945, with the exception of an occasional one or two on an animal.

In 1945, the counts on cattle in the scrub-pasture zone of Margam were restricted to those at Longlands and Graig-Goch Farms. Activity became apparent on these farms in 1945 on 27 March and increased steadily until 13 April when an average of 10·75 and 16·1 ticks per head was recorded at Longlands and Graig-Goch, respectively. In the course of the next 4 days (13–17 April), the ticks on the stock at Longlands rapidly increased and reached their height of activity on 24 April at Longlands and 27 April at Graig-Goch, that is, 17–20 days earlier than in 1944 at the same centres and a fortnight earlier than the corresponding nymphal peak on grassland in 1945 (Fig. 9). The next three successive counts at Longlands showed slight fluctuations with a 'secondary' spring peak of activity on 11 May. From 2 June the tick populations at each centre decreased gradually to the second week in July, thereafter fluctuating at a low level until about mid-August. On Longlands' cattle, the autumn peak was of short duration and not as marked as in 1944. A similar peak also occurred on cattle at Graig-Goch but it attained only about a third of the value of that in the spring.

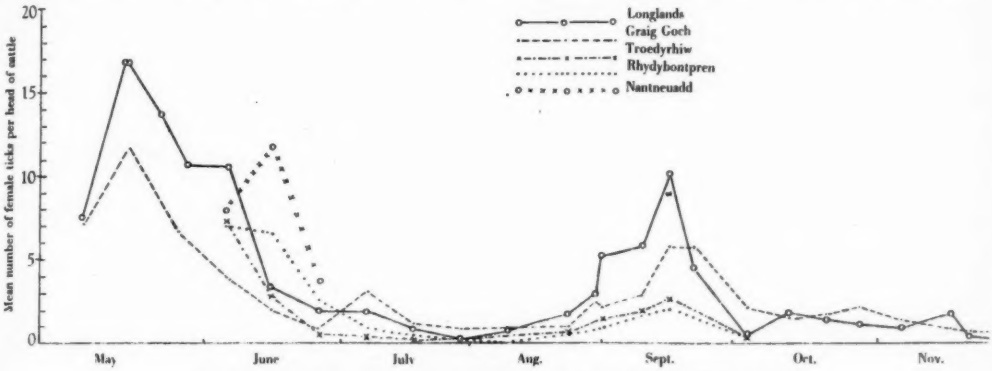


Fig. 8. Female tick activity curves on cattle in Glamorgan (1944).

Table 5 has been included in the present paper with the object of presenting the frequency distribution of ticks on one herd of cattle over a period of 2 years.

(iii) *Seasonal activity of the tick in other parts of Wales.* In 1945, counts, besides those at Longlands and Graig-Goch Farms, were undertaken on cattle at Rudbaxton Farm, Haverfordwest, Pembrokeshire, and surrounding area and in the Cahn Hill neighbourhood to determine whether the seasonal activity of the tick in other parts of Wales shows deviations comparable with those recorded in the north of England and in Scotland. The seasonal activity recorded at Rudbaxton Farm followed the same general plan as that already indicated on cattle in the Margam area of Glamorgan, but the degree of incidence was considerably higher. The mean population of ticks on a unit of twelve cattle at Rudbaxton Farm in the spring peak was 98, and in the autumn 54 (Fig. 10).

At the outset it was realized that the methods of management of cattle vary in different localities in

Table 5. *Frequency distribution of ticks on a herd of cattle over a 2-year period*

$\bar{x}$ = mean number of ticks; n = total number of cattle; v = variance; R = range.

Case 1 (3. v. 44)
$\bar{x}=8{\cdot}25$; $n=8$; $v=15{\cdot}557$; $R=10$
Case 2 (11. v. 44)
$\bar{x}=7{\cdot}25$; $n=8$; $v=9{\cdot}071$; $R=10$
Case 3 (22. v. 44)
$\bar{x}=16{\cdot}25$; $n=8$; $v=14{\cdot}786$; $R=11$
Case 4 (23. v. 44)
$\bar{x}=16{\cdot}25$; $n=8$; $v=31{\cdot}643$; $R=15$
Case 5 (1. vi. 44)
$\bar{x}=13{\cdot}625$; $n=8$; $v=18{\cdot}218$; $R=13$
Case 6 (5. vi. 44)
$\bar{x}=6{\cdot}5$; $n=6$; $v=9{\cdot}90$; $R=9$
Case 7 (7. vi. 44)
$\bar{x}=10{\cdot}428$; $n=7$; $v=16{\cdot}286$; $R=9$
Case 8 (15. vi. 44)
$\bar{x}=3{\cdot}5$; n = 8; $v=1{\cdot}143$; $R=3$
Case 9 (26. vi. 44)
$\bar{x}=2{\cdot}0$; $n=8$; $v=1{\cdot}143$; $R=3$
Case 10 (7. vii. 44)
$\bar{x}=1{\cdot}875$; $n=8$; $v=0{\cdot}696$; $R=2$
Case 11 (18. vii. 44)
$\bar{x}=0{\cdot}75$; $n=8$; $v=0{\cdot}5$; $R=2$
Case 12 (29. vii. 44)
$\bar{x}=0{\cdot}25$; $n=8$; $v=0{\cdot}214$; $R=1$
Case 13 (9. viii. 44)
$\bar{x}=0{\cdot}71$; $n=7$; $v=0{\cdot}938$; $R=2$
Case 14 (23. viii. 44)
$\bar{x}=6{\cdot}6$; $n=8$; $v=0{\cdot}511$; $R=2$
Case 15 (31. viii. 44)
$\bar{x}=14{\cdot}9$; $n=8$; $v=12{\cdot}00$; $R=11$
Case 16 (9. ix. 44)
$\bar{x}=6{\cdot}0$; $n=8$; $v=14{\cdot}286$; $R=10$
Case 17 (22. ix. 44)
$\bar{x}=10{\cdot}5$; $n=8$; $v=7{\cdot}429$; $R=8$
Case 18 (3. x. 44)
$\bar{x}=4{\cdot}75$; $n=8$; $v=4{\cdot}500$; $R=5$
Case 19 (14. x. 44)
$\bar{x}=0{\cdot}5$; $n=8$; $v=0{\cdot}571$; $R=2$
Case 20 (19. x. 44)
$\bar{x}=1{\cdot}5$; $n=8$; $v=4{\cdot}0$; $R=2$
Case 21 (27. x. 44)
$\bar{x}=0{\cdot}76$; $n=8$; $v=0{\cdot}789$; $R=2$
Case 22 (6. xi. 44)
$\bar{x}=1{\cdot}0$; $n=8$; $v=0{\cdot}587$; $R=2$
Case 23 (18. xi. 44)
$\bar{x}=2{\cdot}0$; $n=8$; $v=2{\cdot}286$; $R=5$
Case 24 (25. xi. 44)
$\bar{x}=0{\cdot}25$; $n=8$; $v=0{\cdot}214$; $R=1$
Case 25 (29. xi. 44)
$\bar{x}=0{\cdot}25$; $n=8$; $v=0{\cdot}214$; $R=1$
Case 26 (27. iii. 45)
$\bar{x}=3{\cdot}62$; $n=8$; $v=3{\cdot}39$; $R=6$
Case 27 (6. iv. 45)
$\bar{x}=9{\cdot}375$; $n=8$; $v=6{\cdot}86$; $R=3$
Case 28 (13. iv. 45)
$\bar{x}=10{\cdot}75$; $n=8$; $v=8{\cdot}41$; $R=9$
Case 29 (17. iv. 45)
$\bar{x}=23{\cdot}5$; $n=8$; $v=19{\cdot}98$; $R=14$
Case 30 (24. iv. 45)
$\bar{x}=26{\cdot}0$; $n=8$; $v=87{\cdot}24$; $R=26$
Case 31 (3. v. 45)
$\bar{x}=13{\cdot}5$; $n=8$; $v=19{\cdot}62$; $R=13$
Case 32 (11. v. 45)
$\bar{x}=16{\cdot}37$; $n=8$; $v=8{\cdot}473$; $R=9$
Case 33 (22. vi. 45)
$\bar{x}=13{\cdot}6$; $n=8$; $v=38{\cdot}69$; $R=22$
Case 34 (2. vi. 45)
$\bar{x}=7{\cdot}62$; $n=8$; $v=7{\cdot}90$; $R=9$
Case 35 (12. vi. 45)
$\bar{x}=5{\cdot}88$; $n=8$; $v=13{\cdot}25$; $R=10$
Case 36 (19. vi. 45)
$\bar{x}=3{\cdot}88$; $n=8$; $v=2{\cdot}82$; $R=5$
Case 37 (26. vi. 45)
$\bar{x}=3{\cdot}5$; $n=8$; $v=1{\cdot}99$; $R=4$
Case 38 (4. vii. 45)
$\bar{x}=2{\cdot}62$; $n=8$; $v=1{\cdot}40$; $R=3$
Case 39 (11. vii. 45)
$\bar{x}=1{\cdot}12$; $n=8$; $v=1{\cdot}25$; $R=4$
Case 40 (18. vii. 45)
$\bar{x}=1{\cdot}37$; $n=8$; $v=1{\cdot}40$; $R=4$
Case 41 (23. vii. 45)
$\bar{x}=1{\cdot}75$; $n=8$; $v=1{\cdot}33$; $R=4$
Case 42 (4. viii. 45)
$\bar{x}=1{\cdot}6$; $n=8$; $v=0{\cdot}85$; $R=3$
Case 43 (13. viii. 45)
$\bar{x}=1{\cdot}3$; $n=8$; $v=1{\cdot}14$; $R=3$
Case 44 (20. viii. 45)
$\bar{x}=0{\cdot}75$; $n=8$; $v=0{\cdot}5$; $R=2$
Case 45 (27. viii. 45)
$\bar{x}=1{\cdot}6$; $n=8$; $v=0{\cdot}85$; $R=3$
Case 46 (7. ix. 45)
$\bar{x}=10{\cdot}5$; $n=8$; $v=7{\cdot}429$; $R=8$
Case 47 (14. ix. 45)
$\bar{x}=3{\cdot}5$; $n=8$; $v=6{\cdot}29$; $R=7$
Case 48 (21. ix. 45)
$\bar{x}=0{\cdot}75$; $n=8$; $v=1{\cdot}071$; $R=3$
Case 49 (2. x. 45)
$\bar{x}=1{\cdot}13$; $n=8$; $v=0{\cdot}696$; $R=2$
Case 50 (8. x. 45)
$\bar{x}=2{\cdot}0$; $n=8$; $v=1{\cdot}143$; $R=3$
Case 51 (15. x. 45)
$\bar{x}=2{\cdot}62$; $n=8$; $v=1{\cdot}982$; $R=4$
Case 52 (23. x. 45)
$\bar{x}=1{\cdot}0$; $n=8$; $v=0{\cdot}857$; $R=2$

the Principality and that 'it is because of variations in normal practices that deviations from the standard type of seasonal activity occur' (Cameron, 1939). In 1945 a number of farms in the Cahn Hill area and the surrounding districts were visited to determine the seasonal activity of ticks in localities where farming practices differ from those in South Wales. Unfortunately, due to circumstances beyond the control of the writers, it was only possible to make regular counts at one farm, Hendre Eirian, and to supplement them by spasmodic counts at the other farms. As usual in the case of Hendre Eirian and other farms in the area, cattle were kept indoors during the night and in the well-managed closely grazed fields around the farm buildings during the day-time until mid-May. They were then turned 'out-of-doors' at night and allowed, until November, access to the higher ground, locally called 'ffridd', which is covered with poor grasses and bracken and badly infested with tick. Their access to badly infested pastures is therefore considerably later than is the case in South Wales. From Fig. 10 it is seen that ticks first become apparent on the cattle at Hendre Eirian on 26 May, but their belated appearance may be associated with the late exposure to attack. (Cf. activity in South Wales.) It is thus possible that this difference in seasonal activity of the tick in mid-Wales, when compared with that found in South Wales, is not so much due to divergence of the habits of the tick as to the relative availability of the hosts at the time when these are required.

At Hendre Eirian Farm, tick populations on cattle proved abnormally high for June and July, in comparison with those recorded for South Wales, but in view of the high figures for the autumn peak, they are not surprising. Milne (1945*b*) is of the opinion that the practical application of his findings in the north of England indicate that 'farm stock can have the run of infested land in the normal off seasons of activity without danger of infestation even if the land had not been grazed in the seasons of activity'. The data collected at Hendre Eirian and other farms in mid-Wales indicate that while some reduction in tick infestation may result by grazing in June and July, a complete eradication of the tick would not be accomplished in this manner, owing to its high incidence on farm stock in the

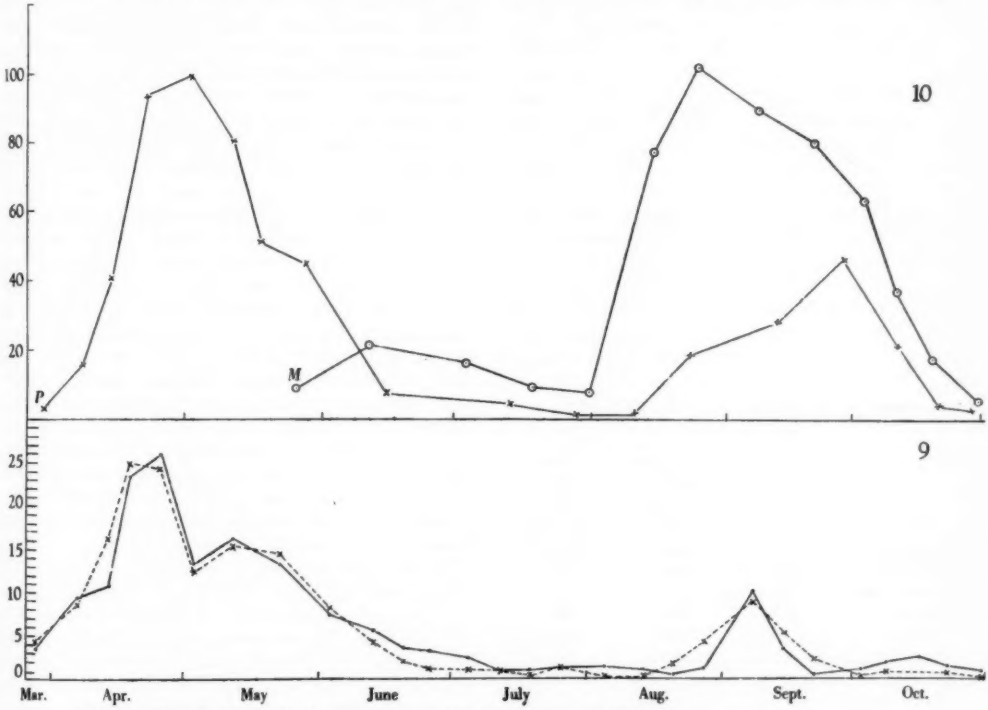


Fig. 9. Female tick activity curves on cattle in Glamorgan (1945).

Fig. 10. Female tick activity curves on cattle at Rudbaxton (*P*) and Hendre Eirian (*M*) (1945).

'off-season' and an abundance of wild animals and birds as hosts at all times of the year. Although the peculiar curve of seasonal activity of the tick recorded in 1945 in the Cahn Hill area and surrounding districts in mid-Wales may be related at Hendre Eirian and other farms to the system of farming involving late exposure of the stock to attack in the spring, nevertheless, the evidence collected on some twenty-one farms where the methods of management of the cattle resemble, in essential details, those practised in the Margam area in South Wales strongly suggests a single peak of activity, attaining its maximum about August and comparable with that in the Hill o' Fare in Aberdeenshire (Hendrick, Moore & Morison, 1938).

(iv) *Engorgement period of female ticks on cattle.* Four batches, each of twenty-five female ticks, whose scuta were marked with cellulose paint, were placed on the flank of each of six different breeds of cattle and allowed to feed, the breeds being Shorthorn, Hereford, Welsh Black, Friesian, Ayrshire and Redpoll. The ticks were examined daily to ascertain the length of time necessary for engorgement and for 'dropping off'. An analysis of the results (Table 6) indicates that the length of the feeding period varied from 7 to 13 days, and that it was not associated in any way with the breed of cattle.

Table 6. *Engorgement period of female ticks on cattle*

Breed	Shortest period of feeding (in hours)	Longest period of feeding (in hours)	Mean period of feeding (in hours)
Shorthorn	168−	264−	216−
Hereford	192−	264−	216+
Welsh Black	168+	312−	216−
Friesian	168−	288−	240−
Ayrshire	180+	276+	264+
Redpoll	168+	264+	228+

(v) *Comparative susceptibility of cattle breeds to tick infestation.* It is sometimes claimed by farmers and others that different breeds of cattle vary in their susceptibility to tick infestation. This has gained some support from the work of MacLeod (1932) who has shown in the case of sheep that, when first exposed, the Blackfaces become more heavily infested than the Cheviots and remain so until May when the numbers of ticks on both breeds gradually decreased. No data are presented, however, regarding age classes or the individual susceptibility of the sheep of the same age class to infestation.

This aspect in relation to breeds of cattle was investigated both in 1944 and 1945. The breeds, Shorthorn, Hereford, Friesian and Welsh Black, were involved in the investigations of 1944 and Redpoll, Ayrshire, Shorthorn, Friesian and Hereford in those of 1945. From an examination of the results of both years (Table 7), it is obvious that there were no marked differences in the degree of susceptibility of the six breeds under consideration and that the seasonal activity of the tick on any particular breed did not show any outstanding variation from that computed from all breeds (Fig. 11). In 1944, the peak for Shorthorns, Herefords and Friesians coincided but the maximum incidence in the case of the Welsh Blacks occurred 14 days later, after which period the decline was more rapid in this breed than in the other three breeds. In 1945, coincident peaks in relation to dates occurred in the case of Ayrshires and Friesians. With the Hereford cattle, on the other hand, the tick attained maximum activity some 7 days earlier as also it did on Redpoll cattle, but on the latter a secondary peak became apparent about 21 days later. The recorded peak may not necessarily be the true peak, for as pointed out by Milne (1945*a*) the true peak may lie, as already mentioned, anywhere within plus or minus half an interval of the recorded peak.

In 1944 the population of ticks was greater on Shorthorns than on Herefords, while in 1945 the reverse was the case. Although these results indicate that there are no significant differences in the susceptibility of these breeds to attack, it must be emphasized that the work of two years is inadequate to make more than tentative statements in this connexion. The details of the counts are given in Table 7.

Table 7. *Mean counts of tick populations on dairy stock around the peak periods* (1944–5)

1944											Total
Friesians	2	18	10	5	4	1	2	0	1	0	43·0
Herefords	5	15	14	17	1	1	1	0	0	0	54·0
Shorthorns	11	18	12	12	3	2	3	1·5	2·5	0·5	65·5
Welsh Blacks	5	15	14	17	1	1	1	0	0	0	54·0
1945											Total
Redpoll	7	10	13	17	14	12	17	5	7	4	106·0
Ayrshire	7	12	13	23	36	15	19	13	7	8	153·0
Shorthorn	1	6	5	20	15	10	12	8	12	4	93·0
Friesians	4	8·6	9·3	26·3	31	15	19·3	16·3	7·0	3·3	140·1
Herefords	2·5	11·5	11·5	24·5	33	15	18	24·5	6·0	9	155·5

(vi) *Tick infestation and reproductive condition of the host.* It has been suggested that the susceptibility of hosts to attack by a blood-sucking ectoparasite, such as the tick, is correlated with their physiological condition. MacLeod (1932) stated that 'the susceptibility of a sheep to [tick] infestation appears to depend on its physiological condition and breed', and with the aid of field experiments he showed that a barren ewe was markedly less infested by female ticks than were two pregnant ewes on the same pasture. Similarly, Rowan & Gregson (1935), indicated that female ticks (*Dermacentor andersoni* Styles), refused to engorge on a rabbit in anoestrus despite the insertion of the mouth parts. When, however, the rabbit was rendered sexually active, subsequent to prolonged exposure to light, the ticks fed readily. It has been shown by Carrick & Bullough (1940), on the other hand, that parasitization of *Erinaceus europaeus* by *Ixodes ricinus* does not appear to be related to the reproductive condition of the host animal and the amount of sex hormone in the host's blood has no apparent effect on the fecundity of the tick.

Table 8. *Tick populations on milking and in-calf cows*

Animal index	No. of ticks per beast: 'Barren' cows	No. of ticks per beast: In-calf cows	Differences in tick populations ($c-d$): +	Differences in tick populations ($c-d$): −	Square of differences ($c-d$)
1	112	64	48	—	2304
2	78	69	9	—	81
3	84	70	14	—	196
4	102	84	18	—	324
5	114	83	31	—	961
			+120		3866

$$\text{S.S.} = 3866 - \frac{120^2}{10} = 2426.$$

Mean difference = 24·0.

$$\text{S.E. of mean difference} = \sqrt{\frac{2426}{4 \times 5}} = 11{\cdot}01.$$

$$t = \frac{24{\cdot}0}{11{\cdot}01} = 2{\cdot}18.$$

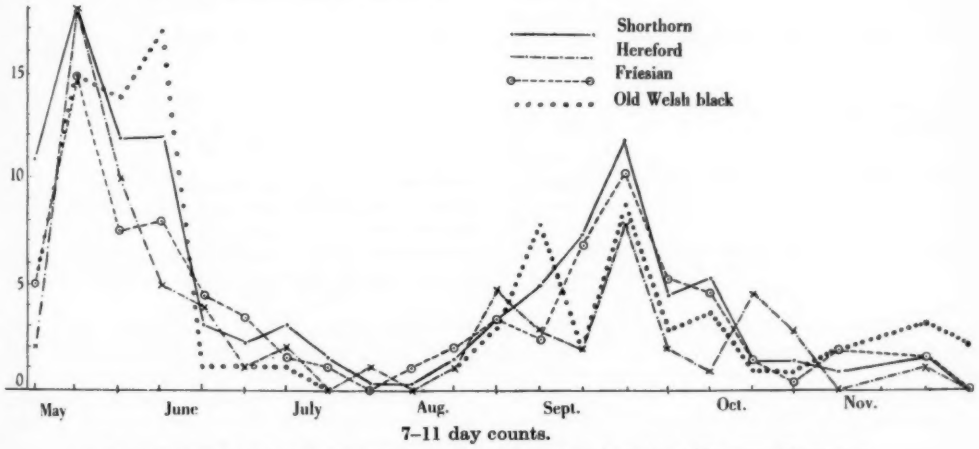


Fig. 11. Female tick activity curves on different breeds of cattle in Margam, Glamorgan.

In the present studies, counts were taken of the number of attached females on cows in calf and on those in a barren condition at Hendre Eirian Farm. In each case, the population indices were determined by the method already indicated. Counts were limited to five cows in each group as only five (in-calf) cows were available at the time for examination (Table 8).

Reference to the table of t shows that for 4 degrees of freedom the probability of exceeding this value purely by chance is greater than 0·1, hence when $P = 0{\cdot}05$ the mean populations are not significant. Counts were also made at Troed-yr-rhiw and Morfa Bach Farms, Glamorgan, on young stock and dairy cattle which had been grazing together on the same pastures for a similar period (Table 9).

In this case the sum of the squares = 360·55 with mean difference = 0·35. Since the standard error of the mean difference $= \frac{360{\cdot}55}{19 \times 20} = 0{\cdot}95$ then $t = 0{\cdot}37$. For 19 degrees of freedom the probability of exceeding this value by chance is greater than 0·6, proving that the mean difference between milking and non-milking stock is again not significant.

From the cattle at Hendre Eirian Farm, ten fully gorged ticks were carefully removed from each cow and transferred to the laboratory. Here the engorged ticks from each cow were kept separately

Table 9. *A comparison of tick density on stock at Troed-yr-rhiw and Morfa Bach Farms, Margam* (1944)

Date			No. of ticks per beast				
			1	2	3	4	5
2 June	Troed-yr-rhiw	Young stock	9	14	10	12	15
	Troed-yr-rhiw	Dairy stock	10 (2)*	11 (3)	9 (3)	17 (3)	12 (3)
12 June	Troed-yr-rhiw	Young stock	9	12	8	7	14
	Troed-yr-rhiw	Dairy stock	12 (2)	9 (3)	10 (3)	11 (2)	12 (3)
2 June	Morfa Bach	Young stock	23	17	18	20	7
	Morfa Bach	Dairy stock	21 (2)	15 (2)	22 (2)	8 (2)	15 (2)
15 June	Morfa Bach	Young stock	18	21	21	24	23
	Morfa Bach	Dairy stock	20 (2)	19 (3)	14 (2)	27 (2)	19 (1)

* Figures in parenthesis denote first, second or third calvers.

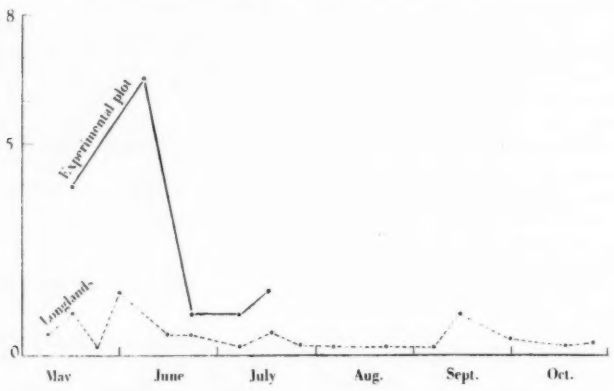


Fig. 12. Female tick activity curves on sheep in Margam, Glamorgan.

on moist filter paper in Petri dishes together with fifteen males to ensure fertilization. Egg-laying commenced 27–35 days after the ticks had been removed from their hosts. The mean number of eggs laid by females, derived from the two groups, (*a*) barren cows and (*b*) in-calf cows, is presented

Table 10. *Number of eggs laid by ticks from* (*a*) *'barren' cows, and* (*b*) *in-calf cows*

Tick sample	No. of eggs laid by female ticks removed from	
	(*a*) Barren cows	(*b*) In-calf cows
1	984	974
2	913	832
3	1012	898
4	1625	2051
5	879	1076
Mean	$\frac{5413}{5}=1082{\cdot}6$	$\frac{5831}{5}=1166{\cdot}2$

in Table 10. The difference in the arithmetical mean of the eggs laid in each group is only six per cent, from which it would appear that the sexual condition of the host does not influence the fecundity of the tick, a view which gains some support from the work of Carrick & Bullough (1940).

(vii) *Seasonal prevalence of the tick on sheep.* Sheep grazing infested land were only available for critical and repeated examination at two centres in South Wales and the number of ticks found on them in both years under review was practically negligible. The sheep enclosed in 1944 on an area known to be heavily infested on Margam Moors, gave an average on each sheep of 4·0 ticks in mid-May and 6·4 ticks in the first week of June. At Longlands Farm, sheep which were allowed to run the same grazings as badly infested cattle did not show a heavy infestation either in 1944 or 1945. The ticks rarely exceeded one per sheep at any time during either season (Fig. 12).

(viii) *Seasonal prevalence of the tick on horses.* The nostrils and lips of the three horses, previously mentioned, on Margam Moors were found during the 'spring' months densely covered with gorging nymphs. In addition, gorging females were present in the groin, tail region and between the front and hind legs. In 1945 counts were carried out on these horses from the end of March to mid-July and from

the necessarily scanty details given in Table 11, it will be noted that the peak of activity in spring corresponds closely to that recorded on cattle. The presence of gorged females on the horses on the date of the initial count suggests that the first females were active sometime before 27 March, at least in 1945.

Table 11. *Female tick populations on horses* (*Margam Moors*), 1945

Date	2-year old		1-year old
	A	*B*	
27 March	3	3	4
6 April	5	7	13
13 April	10	13	28
17 April	25	23	26
24 April	15	15	9
3 May	10	18	17
11 May	19	10	10
22 May	5	9	8
2 June	7	4	7
12 June	5	3	8
19 June	8	2	3
26 June	1	1	3
4 July	2	0	5
16 July	1	1	0

4. SUMMARY

A consideration of the tick-activity data for South Wales reveals that there are usually two peaks of female activity in this region, one occurring in spring, the other in autumn. On an experimental area, divided into eight plots, on the Margam Moors, Glamorgan, significantly varying population levels are present on the different plots, although the peak of activity on all of them is attained around the same period. The seasonal activity of the nymphal and larval forms in South Wales is essentially comparable to that recorded by Milne (1945*a*) in the north of England. There is reason to believe that in mid-Wales a single summer peak of tick activity exists.

The engorgement period of female ticks on cattle varies from 7 to 13 days. The tick shows a distinct preference for the hind region of cattle for feeding purposes. The differences in the mean populations of ticks on different breeds of cattle are not statistic ally significant, at least, on the six breeds tested in 1944 and 1945. In the case of cattle, tick density and fecundity of the female are not correlated with the physiological condition of the host.

In South Wales, the populations of ticks on sheep are significantly lower than those on cattle, even where both hosts are pastured on the same ground for similar periods. The seasonal activity of female ticks on horses follows the same general trend as that noted on cattle.

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REFERENCES

ARTHUR, D. R. (1945). *Nature, Lond.*, **153**, 538.
ARTHUR, D. R. (1946). *Parasitology*, **37**, 154.
CAMERON, A. E. (1939). *Trans. Highl. Agric. Soc. Scot.* **51**, 136.
CARRICK, R. & BULLOUGH, W. S. (1940). *Parasitology*, **32**, 313.
FALKE, H. (1931). *Z. Morph. Ökol. Tiere*, **21**, 567.
HENDRICK, J., MOORE, W. & MORISON, G. (1938). *Nature, Lond.*, **141**, 648.
MACLEOD, J. (1932). *Parasitology*, **24**, 382.
MACLEOD, J. (1939). *Bull. Ent. Res.* **30**, 103.
MILNE, A. (1943). *Ann. Appl. Biol.* **30**, 240.
MILNE, A. (1945*a*). *Parasitology*, **36**, 142.
MILNE, A. (1945*b*). *Parasitology*, **36**, 153.
MILNE, A. (1945*c*). *Ann. Appl. Biol.* **32**, 128.
ROWAN, W. & GREGSON, F. D. (1935). *Nature, Lond.*, **135**, 652.
ROWLANDS, W. T. (1945). *Vet. Rec.* **57**, 489.
TOTZE, R. (1933). *Z. vergl. Physiol.* **19**, 110.
WHELER, E. G. (1899). *Proc. R. Agric. Soc. Engl.* **4**, 626.

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DIPETALONEMA EVANSI (LEWIS, 1882) AND ITS MICROFILARIA FROM *CAMELUS DROMEDARIUS*

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(With 11 Figures in the Text)

CONTENTS

	PAGE		PAGE
Introduction	86	Description of the microfilaria	91
Material and technique	86	Table of the facts recorded by different authors concerning this microfilaria	92
Historical	86	References	92
Description of the adults	90		

INTRODUCTION

The two filarial Nematodes known to parasitize dromedaries in Egypt are *Dipetalonema evansi* and *Onchocerca fasciata*, of which the first is the more common. Representatives of these genera also infect man and other animals and are known to be very common in endemic areas. Doubt has always arisen in the researches of those few who worked on this subject, as to the connexion of the adult *Dipetalonema evansi* with the microfilariae found in the blood of *Camelus dromedarius* and *C. bactrianus*.

It is well known that the microfilariae of species of *Onchocerca* in man and animals are found in the tissues surrounding the habitat of the adult worms, and very seldom, if ever, in the blood-stream. On the other hand, microfilariae of species of *Dipetalonema* are found in the blood. Therefore the only conclusion to be drawn in this case is that the microfilariae found in the blood of dromedaries belong to *D. evansi*.

The aims of this paper are (1) to establish definitely the identity of the Egyptian adult worms and their relation to the filarial embryos found in the blood; (2) to amplify and correct the very few descriptions known both of the adult and the microfilaria of this species; (3) to make a comparative study of presumably the same species recorded by other authors under different names; (4) to report on an interesting case of the occurrence of the adults in the right auricle of a dromedary, apparently a hitherto unrecorded habitat.

MATERIAL AND TECHNIQUE

Adult worms were obtained from the right auricle of a Bishari dromedary, whose death had been caused by them, at Kom-Ombo, Upper Egypt. On postmortem the worms were found to be intricately coiled together, forming a mass the size of a golf-ball. They were separated with difficulty and were found to number 216. Of these 83 were males and 133 females. Other Nematodes of this species were obtained from the blood-vessels of the testicles and lungs of dromedaries from the Cairo abattoir.

Microfilariae were obtained from the blood of dromedaries from the Cairo abattoir as follows:

The blood of the slaughtered animals was collected in citrated tubes. On arrival at the laboratory it was distributed in test-tubes, laked with fresh water to which a few drops of acetic acid had been added, and left to sediment. The sediment was obtained by decanting the supernatant fluid into centrifuge tubes, adding fresh water only and spinning at a high speed. The sediment was again washed with fresh water to get rid of any trace of the acid and the citrate, and the centrifugalization repeated. The contents of the lowermost part of the centrifuge tube were homogeneously distributed on several glass slides and dried with the aid of gentle heat. In this way the author was able to collect a few hundred microfilariae in clear fields and concentrated on a few glass slides. Giemsa and Leishman stains were used with good results.

Other ordinary blood-films were obtained containing microfilariae from different sources.

HISTORICAL

The microfilaria of this species was first discovered in the blood of Indian dromedaries by Evans, and described by Lewis in 1882 under the name of *Filaria evansi*, in honour of its discoverer.

Edmond & Étienne Sergent (1905) found in the blood of Algerian dromedaries the embryos and the adults of a filaria which they presumably identified as *F. evansi*. They found that 4–5 % of these dromedaries taken at random were infected with this

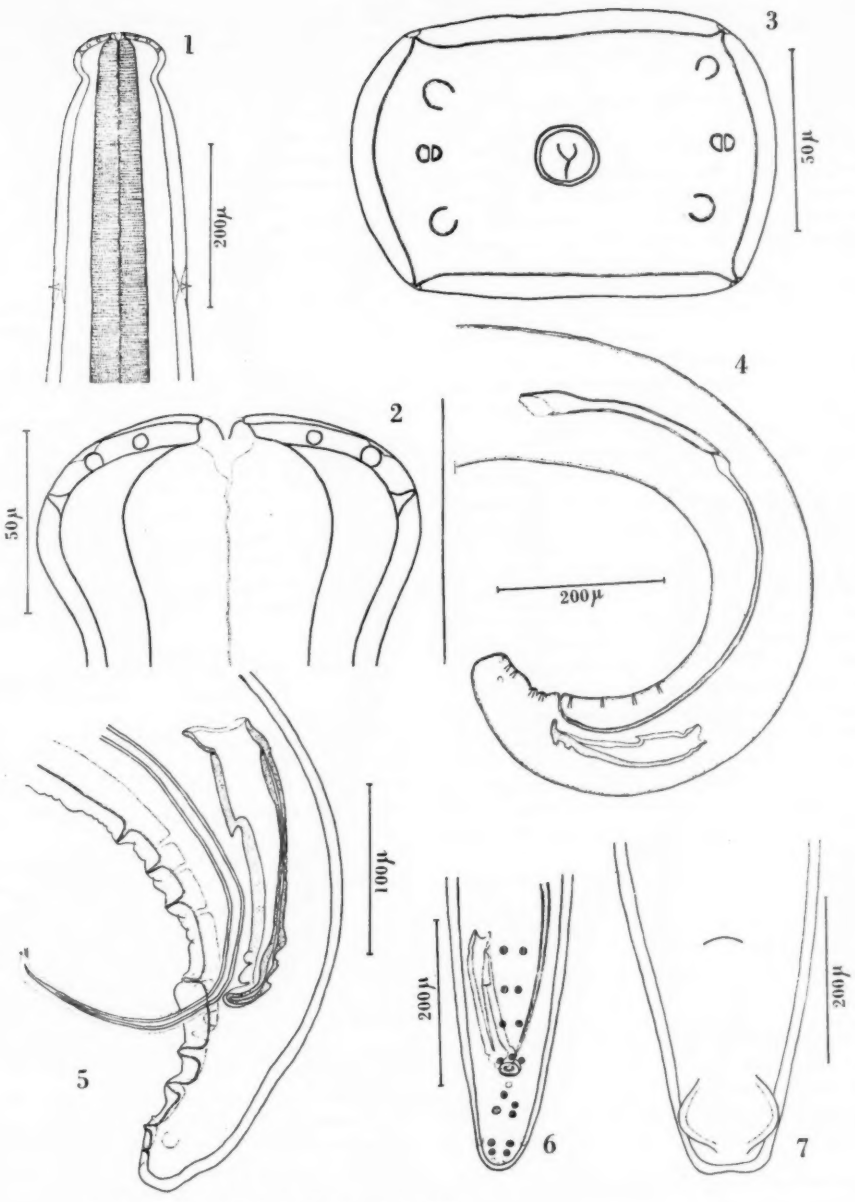

Fig. 1. Anterior end of adult, ventral view.
Fig. 2. Head end of adult, ventral view.
Fig. 3. Head end, shaved off with a razor and mounted anterior end upwards.
Fig. 4. Posterior end of male, left lateral view.
Fig. 5. Posterior end of male, left lateral view.
Fig. 6. Posterior end of male, ventral view.
Fig. 7. Posterior end of female, ventral view.

microfilaria. The interruptions they mention in their description of the microfilaria correspond to the nerve-ring, excretory vesicle, internal mass and anal pore.

Mason (1906) observed in camels' blood in Egypt a microfilaria which he, in a later publication, presumes to be *F. evansi*. The latter publication mainly deals with the symptoms, the pathology, the etiology and the possible vector of the infection.

Cazalbou (1906) gives a short description of a microfilaria which he designates *Mf. sanguinicole* and which he found in Tombouctou at the same time in a dromedary, a donkey and a bull. He did not see the adult filaria and his description of the microfilaria, examined only in the fresh blood, does not permit identification. In any case, the microfilariae in the three hosts mentioned by him, according to our present knowledge, cannot have belonged to the same species.

Balfour (1911) described and figured a Leishman-stained microfilaria from the blood of dromedaries in the Korora Province of the Red Sea, Sudan. He gave it the name of *Mf. camelensis*. The positions of the landmarks he gives in microns are converted into percentages of the average length of the worm from the anterior end, in a table below. These positions represent the cephalic space, nerve-ring, excretory vesicle and anal pore respectively.

Pricolo (1913), working in Tripoli, gave a meagre description of a microfilaria which he obtained from the blood of Tunisian and Erythrean dromedaries. According to this author the periodicity of this microfilaria is nocturnal. In a later publication in the same year Pricolo obtained the adults of this filaria from the blood-vessels of the lungs and the testicles, and proposed the name *Filaria haematica cameli* for them.

Yakimoff and collaborators (1916) studied a sheathed microfilaria in the blood of camels, *Camelus bactrianus*, from Russian Turkestan. They found the average percentage of infection among these animals to be nearly 9%. The landmarks they describe in this worm, and their positions in microns, are translated into percentages of the average length of the worm from the anterior end, in the table below. They represent the cephalic space, nerve-ring excretory vesicle and anal pore respectively. These authors also describe the internal mass and call it a gastro-intestinal canal.

Owing to a misinterpretation in the interruptions of the column of nuclei recorded for *Mf. evansi* by the Sergents and for *Mf. camelensis* by Balfour, Yakimoff *et al.* came to the conclusion that their microfilaria resembled *Mf. camelensis*. In fact, the table of comparison given by these latter authors of the two supposed species shows that the interruptions compared do not correspond to each other. The mistake in the table is due firstly to the fact that the interruptions quoted for the Sergents do not include the cephalic space, while in Balfour's and Yakimoff's microfilariae the length of the cephalic space is mentioned and considered as the first interruption, and therefore compared with the first interruption of the Sergents, which is in fact the position of the nerve-ring. Secondly, the Sergents include the position of the internal mass in their description, and it is considered by them as the third interruption. This organ is not included in Balfour's or Yakimoff's account.

Consequently the second, third and fourth interruptions of the Sergents, which correspond to the positions of the excretory vesicle, the internal mass and the anal pore, are erroneously compared with the positions of the nerve-ring, the excretory vesicle and the anal pore respectively of the microfilariae described by Balfour and by Yakimoff. Furthermore, the figure given by Yakimoff *et al.* for the position of the second interruption (*e.v.*) in the Sergents' worm should be 88·75μ from the anterior end, and not 83·75μ as stated by them, because the Sergents state that it is 35·5% of a 250μ long worm. Accordingly the table of comparison of Yakimoff *et al.* should be as follows:

	Microfilaria of the Sergents	Microfilaria of Balfour	Microfilaria of Yakimoff
Cephalic space	—	10	5·5–12
Nerve-ring	55	45	38–56
Excretory vesicle	88·75	65	60–77
Anal pore	225	190–207	177–184

All measurements in the above table are in microns.

According to Yakimoff *et al.* this subject has been discussed in the *Report of the Bureau of Microbiology of New South Wales* in 1909, and the microfilaria found in the blood of camels of Western Australia resembles *Mf. evansi*.

Bouin (1921) found in South Morocco a microfilaria in the blood of camels which he considers to be the embryo of *Filaria evansi*. In one of these animals he recovered the adults and identified them as *F. evansi*.

Romanovitch (1916) described under the name *Deraïophoronema cameli* a Nematode from the pulmonary tissue of the camel, *Camelus bactrianus*, from the Kirghiz Plains, Russia. His description is based on two males, one complete and the other without the tail end, and a female in shreds. The short description given corresponds to that of *Dipetalonema evansi*.

Baylis & Daubney (1923) published the first intelligible account of the morphology of the adult worm, which was referred by them to the genus *Acanthocheilonema* Cobbold, 1870. Their description

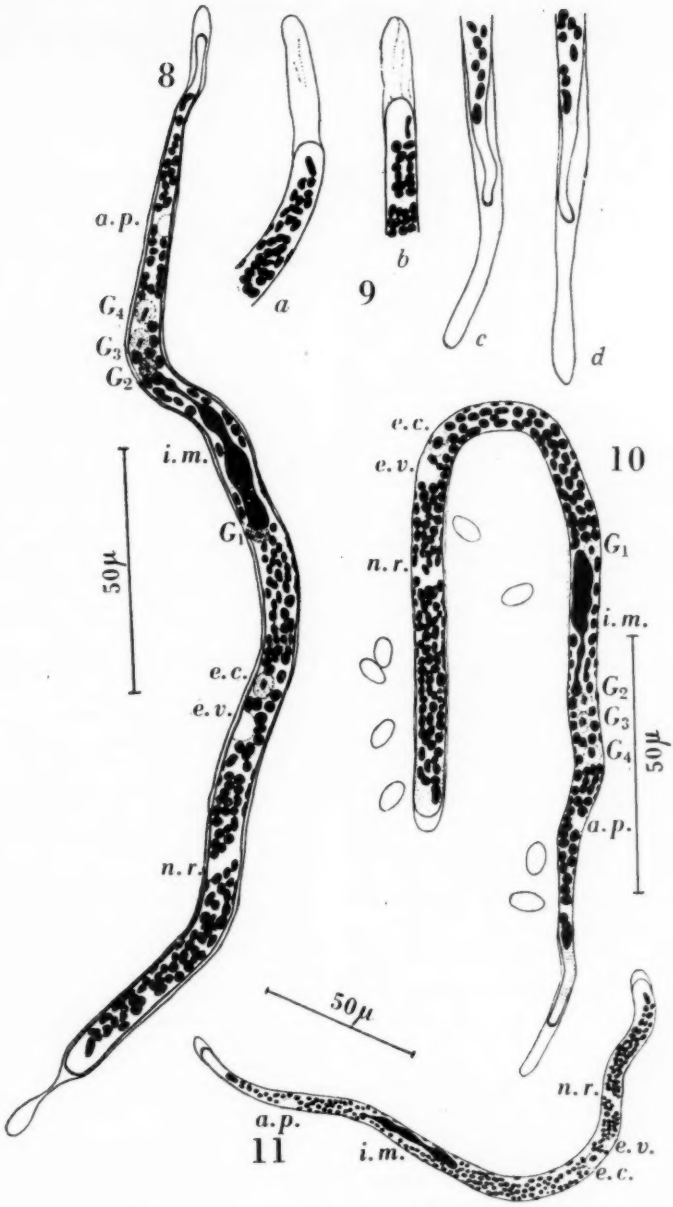

Fig. 8. *Microfilaria evansi* from the blood of *Camelus dromedarius.*
Fig. 9. Two anterior (*a*, *b*) and two posterior (*c*, *d*) ends of same.
Fig. 10. *Mf. evansi* from *Camelus dromedarius.*
Fig. 11. *Mf. evansi* from *Camelus dromedarius.*

All the figures are camera lucida drawings made by the author from nature.

is based on some rather fragmentary material received from the Zoological Survey of India, and partly on notes and drawings supplied by Major Stewart.

Boulenger (1924), while in the Punjab, examined a number of well-preserved specimens from the collection of the Lahore Veterinary College, and described them accurately, provisionally retaining the species in the genus *Acanthocheilonema*.

Foley, Catanei & Vialatte (1926) found a microfilaria in the blood of Algerian dromedaries which they believed to be *Mf. evansi*. The positions of the three landmarks they give in microns are converted into percentages of the average length of the worm from the anterior end, in the table below. They represent the nerve-ring, excretory vesicle and anal pore respectively.

Yorke & Maplestone (1926) consider the genus *Acanthocheilonema* as a synonym of *Dipetalonema*, and in their definition of the genus state that the embryos are unsheathed.

In a later publication (1939) Baylis refers this species to the genus *Dipetalonema* Diesing, 1861. In his definition of this genus Baylis, as did Yorke & Maplestone, includes 'microfilariae without sheath'; this should be emended as follows, 'microfilariae with or without sheath'.

DESCRIPTION OF THE ADULTS

The worms are creamy white in colour, with a smooth cuticle that appears to be devoid of any special characteristics or even transverse striations.

The head end of this filaria, if seen from the dorsal or the ventral surface, is distinctly broader than the part immediately posterior to it. When viewed laterally the head appears rounded and gradually narrows anteriorly. In the female the head measures 113–128μ in breadth when viewed ventrally, and only 90μ when viewed laterally.

The oral opening is provided with a cuticular shield and is simple and rounded when viewed from the anterior end. It leads into a vestigial, poorly chitinized oral cavity.

The head-papillae are arranged in two circles, the outer one being composed of four conical papillae, two dorsolateral and two ventrolateral. The inner circle is composed of eight ear-shaped papillae, four of which are situated just internal to the papillae of the outer circle. The remaining four are D-shaped and are arranged in pairs laterally, one pair to the right and the other to the left. This description and the accompanying figure of the head-papillae are based on a preparation made by shaving a thin slice off the anterior end of a female with a safety razor and mounting it in glycerin-gelatin, anterior end upwards.

Ventrally or dorsally viewed, only three papillae are seen on each side of the oral opening and situated at approximately equal distances from each other. The outermost represent the papillae of the external circle and can be easily recognized on account of their conical shape. The middle papillae represent two of the four peripheral papillae of the internal circle, the innermost the lateral D-shaped papillae.

There is a pair of lateral cervical papillae situated close to the anterior end, at about the junction of the anterior and middle thirds of the muscular part of the oesophagus, in one male at 315μ from the anterior end.

The male measures, in the material at hand, from 8 to 11 cm. in length and 248–405μ in maximum breadth. The diameter of the head varies from 105 to 122μ. The oesophagus is composed of an anterior muscular and a posterior granular part. Its total length ranges from 6 to 6·9 mm. in length; the muscular part of this is from 616 to 968μ in length. The posterior end of the male is much narrower than the anterior and shows a few coils simulating a vine tendril. The tail here measures 99–135μ in length, and has a rounded tip. There are no caudal alae.

The caudal papillae are all pedunculated and consist of two rows of subventrally situated, more or less symmetrical papillae. There are four pairs of equidistant pre-cloacal papillae with an extra single median papilla at the anterior rim of the cloacal aperture and between the last pair of these, making a total of nine. The post-cloacal papillae comprise four pairs of prominent papillae arranged in two groups of four each, with a space between them equal to the area occupied by one of these groups. The first pair are asymmetrically placed; the rest more or less symmetrical. There is an indication of the presence of a single median papilla immediately behind the posterior rim of the cloaca and anterior to the first post-cloacal pair. Three similar stalked papillae can be made out at the extreme posterior end of the tail. Thus the post-cloacal papillae are twelve in number. Another structure which cannot be overlooked is a pair of small ear-shaped ventrolateral cuticular lobes situated opposite to the third pair of papillae. The above description of the head and caudal papillae augments and modifies the descriptions of these structures given by Baylis & Daubney and by Boulenger.

The spicules are unequal in length and dissimilar in shape. The short right spicule measures 168–252μ, the majority 203μ in length. It is thick and provided with a few protuberances, especially towards its distal end. Its posterior tip is rounded, and there is a sharp hook-shaped elevation on the ventral surface, at about the junction of the first and second thirds of its length. The cavity of the spicule is conical in shape. The long left spicule is thin and is composed of two parts welded together, a thicker and shorter proximal tubular part which ranges in

length between 231 and 315μ, and a longer thinner spatulate part distally which measures 420–653μ in length. The whole spicule measures 588–946μ in length and its tip gradually narrows to a point. It is covered distally with a membranous sheath possessing a quadridigitate tip. No gubernaculum could be detected in this species.

The female is longer and stouter than the male. In contrast to the condition in the male, it is difficult to distinguish between the anterior and posterior ends with the naked eye, on account of their almost equal breadth. The body measures 14·5–18·5 cm. in length and 616–792μ in maximum breadth. The oesophagus measures 6·436–7·052 mm. in length, of which the anterior muscular part measures 540–792μ. The union of the muscular and granular parts of the oesophagus is immediately posterior to the opening of the vulva, which is situated at 495–686μ from the head end. The anal opening in the female is situated at 279–440μ from the tail end, which is provided with comparatively large ventrolateral cuticular flaps.

DESCRIPTION OF THE MICROFILARIA

When properly collected and fixed this microfilaria shows graceful curves in the blood or may be almost straight. It is sheathed, although the sheath is difficult to make out in Giemsa-stained preparations; hence the failure of some observers to note its presence. It can only be seen at the anterior and posterior ends or at either of them, owing to its close-fitting character on the body. In a specimen that measures 230μ the sheath extends 18μ anterior to the cephalic end and 52μ posterior to the caudal end. In another that measures 254μ, it protrudes anteriorly for 28μ and posteriorly for 34μ.

The size of this microfilaria ranges from 200 to 315μ in length and from 6 to 9μ in maximum breadth, not including the sheath. The cephalic end is rounded, and has the same diameter as the succeeding part of the body. The cuticle shows very fine transverse striations which can be seen best at the tail end. The column of nuclei which stains violet with Giemsa is composed of well-defined elements and is 3–4 nuclei in breadth. They are either rounded or ovoid, with the exception of the first in the cephalic (Mundgebilde) and the last in the caudal ends, which are elongated, measuring twice or three times as much as the ordinary nuclei. This last character has not been recorded by previous observers. Occasionally the column begins with only two parallel rows of about 14μ long, each composed of a file of single nuclei instead of the usual three or four. The last nuclei form a single row of about six in number, the last two or three of which are usually separated from the others by a short space.

Both ends of the body are devoid of nuclei. The length of the cephalic space ranges from 4 to 10μ, and this constitutes 1·5–4·4% of the total length of the body. The length of the non-nucleated part of the tail measures 12–22μ, or 4·3–8·7% of the total length of the body. The tail end shows a gentle bend in its course, and a gradual narrowing towards its rounded tip, which is also slightly broader than the preceding part and is not pointed as has been stated by some authors. The tip of the tail also possesses a slightly thickened cuticle.

The microfilarial landmarks can be seen, even with a low power of the microscope, in the form of interruptions in the column of nuclei as follows:

(1) The nerve-ring (*n.r.*), the first interruption in the column, is shown in the form of an oblique or transverse non-nucleated band situated at 19–30·4% of the length of the body from the anterior end.

(2) The excretory vesicle (*e.v.*), sometimes referred to as the 'V' interruption, is ventral in position and is not far behind the nerve-ring. It is situated at 25·4–39·8% of the length of the body from the anterior end.

(3) The excretory cell (*e.c.*) is close behind the excretory vesicle and cannot always be made out.

(4) The anal pore (*a.p.*) is in the form of a ventral interruption at 13–20·6% of the total length from the posterior end of the body.

(5) The internal mass (*i.m.*) stains pinkish in contrast to the column of nuclei, which stains violet with Giemsa. It is an elongated body with wavy outlines forming constrictions, and is thicker or broader anteriorly. The anterior part occupies about three-quarters of the breadth of the body. Very often the internal mass is divided into two parts connected together by a thin strand of material. The length of this mass ranges from 34 to 44μ, or 12–16·2% of the length of the worm. The internal mass is situated in the posterior half of the body, at 53·2–61·9% of its length from the anterior end. Roughly speaking, it occupies the third quarter of the length of the microfilaria.

(6) The so-called genital cells (*G*1–*G*4) are extremely difficult to make out and are probably situated as follows:

*G*1 is close to the anterior end of the internal mass.

*G*2 is close to the posterior end of this mass, and both this and *G*1 are often masked by the two extremities of this organ.

*G*3 and *G*4 are found closely posterior to *G*2.

The above facts relating to the position of the internal mass and the so-called genital cells are here published for the first time.

Table 1 summarizes the facts recorded by different authors concerning this microfilaria. It will be noted that it has been described under more than one name.

Table 1

Authority	The Sergents	Mason	Cazalbou	Balfour	Pricolo	Yakimoff *et al.*	Bouin	Foley *et al.*	Present author
Date	1905	1906, 1911	1906	1911	1913	1916	1921	1926	
Host	*C. drom.*	*C. drom.*	*C. drom.*, *Equus asinus*, *Bos taurus*	*C. drom.*	*C. drom.*	*C. bactr.*	*C. drom.*	*C. drom.*	*C. drom.*
Locality	Algeria	Egypt	Tombouctou	Korara Pr., Red Sea, Sudan	Tunisian and Erythrean droms. from Tripoli	Russian Turkestan	South Morocco	Algeria	Egypt
Name given to microfilaria	*Mf. evansi*	Presumably *Mf. evansi*	—	*Mf. camelensis*	*Filaria haematica cameli*	*Mf. camelensis*	*Mf. evansi*	Presumably *Mf. evansi*	*Mf. evansi*
Sheath	—	—	—	—	—	Present	Present	Present	Present
Length	250	172	250	220–237	298	184–226	250	186–218	200–315
Maximum breadth	8–10	5	6	—	6	5·7	5–6	5–6	6–9
Cephalic space	—	—	—	4·3 %	—	2·9–5·8 %	—	—	1·5–4·4 %
Nerve-ring	22 %	—	—	19·6 %	—	18·5–27·8 %	—	16–27·4 %	19–30·4 %
Excretory vesicle	35·5 %	—	—	28·4 %	—	31·2–45·3 %	—	26·9–38·3 %	25·4–39·8 %
Anal pore	90 %	—	—	87 %	—	85·4–95·2 %	—	71·7–93·8 %	79·4–87 %

All measurements in this table are in microns.
The percentages are all of the length of the microfilaria from the anterior end.
— denotes not mentioned.

REFERENCES

BALFOUR, A. (1911). *Fourth Report Wellcome Tropical Research Laboratories*, p. 350.

BAYLIS, H. A. (1929). *A Manual of Helminthology, Medical and Veterinary*. London.

BAYLIS, H. A. (1939). *The Fauna of British India, including Ceylon and Burma*. Nematoda, II. London.

BAYLIS, H. A. & DAUBNEY, R. (1923). *Rec. Ind. Mus.*, Calcutta, XXV, 551.

BAYLIS, H. A. & DAUBNEY, R. (1926). *A Synopsis of the Families and Genera of Nematoda*. London.

BOUIN, —. (1921). *Bull. Soc. Centr. Méd. vét.* **74**, 464.

BOULENGER, C. L. (1920). *Proc. Zool. Soc. Lond.* p. 494.

BOULENGER, C. L. (1924). *Parasitology*, **16**, 419.

CAZALBOU, L. (1906). *Bull. Soc. Centr. Méd. vét.*, **60**, 596.

CHITWOOD, B. G. & CHITWOOD, M. B. (1937–8). *An Introduction to Nematology*. Section I, part II.

DUJARDIN, F. (1845). *Histoire Naturelle des Helminthes ou Vers intestinaux*. Paris.

FOLEY, H., CATANEI, A. & VIALATTE, CH. (1926). *Arch. Inst. Pasteur Algér.* **4**, 485.

LEWIS, T. R. (1882). *Proc. Asiat. Soc. Beng.* p. 63.

MASON, F. E. (1906). *J. Comp. Path.* **19**, 118.

MASON, F. E. (1911). *J. Comp. Path.* **24**, 329.

NEVEU-LEMAIRE (1936). *Traité d'Helminthologie Médicale et Vétérinaire*. Paris.

PRICOLO, A. (1913). *Zbl. Bakt.* I, Orig. **67**, 478.

PRICOLO, A. (1913). *Zbl. Bakt.* I, Orig. **71**, 199.

RAILLIET, A., HENRY, A. & LANGERON, M. (1912). *Bull. Soc. Path. exot.* **5**, 392.

ROMANOVITCH (1916). *C.R. Soc. Biol.*, Paris, **79**, 745.

SERGENT, EDMOND & SERGENT, ÉTIENNE (1905). *C.R. Soc. Biol.*, Paris, **58**, 672.

YAKIMOFF, W. L. *et al.* (1916). *Bull. Soc. Path. exot.* **9**, 219.

YORKE, W. & MAPLESTONE, P. A. (1926). *The Nematods Parasites of Vertebrates*. London.

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ANDREPIGYNOTAENIA HAEMATOPODIS, N.G., N.SP., A NEW PROTOGYNOUS TAPEWORM FROM THE OYSTERCATCHER *HAEMATOPUS OSTRALEGUS OCCIDENTALIS* NEUMANN

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(With 17 Figures in the Text)

The material used for the following description consisted of three worms obtained from the intestine of the oystercatcher, *Haematopus ostralegus occidentalis* Neumann, shot near Aberystwyth. This is the first record of a protogynous tapeworm from Britain. In the whole of the Cestoda the protogynous condition is known only in four other genera, namely, *Progynotaenia* Fuhrmann (1909), with six species, and *Proterogynotaenia* Fuhrmann (1911), *Leptotaenia* Cohn (1901) and *Gynandrotaenia* Fuhrmann (1936, 1936*a*), each with a single species. The six known species of the genus *Progynotaenia* have been recorded from Egypt and the White Nile, *Proterogynotaenia rouxi* Fuhrmann from the Aru Islands off the coast of New Guinea, *Leptotaenia ischnorhyncha* (Lühe) from the Mediterranean and *Gynandrotaenia stammeri* Fuhrmann from Germany.

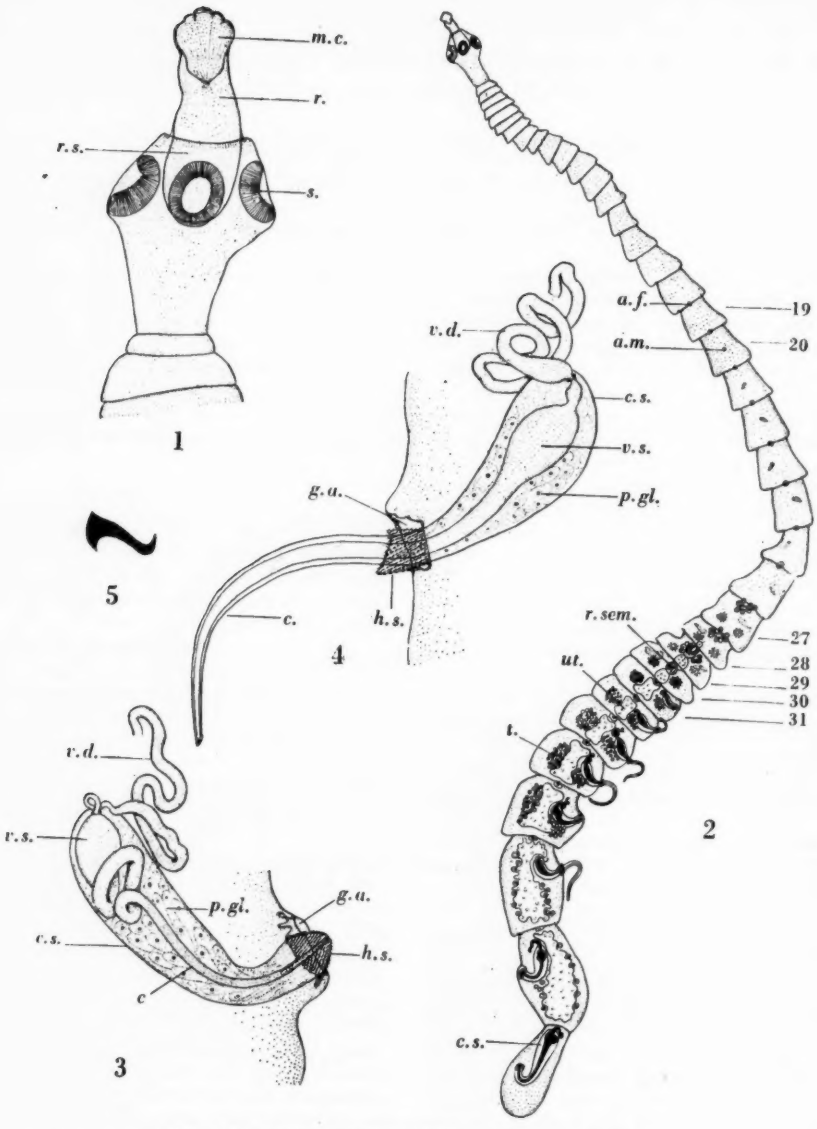
Fuhrmann (1909*a*) separated the cestodes of birds into two fairly distinct groups, a European and North African group and a South American group. In relation to this separation it is extremely interesting to note that *Andrepigynotaenia haematopodis*, in spite of occurring in Britain, is more closely related to the South American *Proterogynotaenia rouxi* than it is to the European and North African genera and species.

THE ANATOMY OF *ANDREPIGYNOTAENIA HAEMATOPODIS*

Only one scolex was available for examination (Fig. 1). It measures 0·41 mm. in breadth and bears four acetabula, each measuring 0·15 mm. in diameter. At the anterior extremity of the scolex there arises an extremely prominent rostellum 0·3 mm. in length. The rostellum is connected posteriorly with a rostellar sac embedded in the anterior part of the scolex and measuring 0·18 mm. in diameter. At its distal extremity the rostellum dilates to a diameter of 0·15 mm., this terminal dilatation is caused by the presence of a well-defined muscular cone embedded in the anterior end of the rostellum with its apex directed backwards. The muscle-fibres, of which the cone is mainly composed, converge towards its apex and then become continuous with the longitudinal muscles of the rostellum itself. The scolex is devoid of hooks, but the writers would hesitate to state that hooks are absent in the species. The late Professor Fuhrmann, in a letter to the writers, suggested that the hooks might have been lost, basing his view on the fact that the rostellum is so strongly developed that it would be expected to be armed.

STROBILA

There is no neck, segmentation beginning immediately behind the scolex. The longest strobila found was complete, and measured 17 mm. in length, 1·32 mm. in maximum breadth and consisted of thirty-eight segments (Fig. 2). The outstanding feature of the strobila is the fact that the female genital organs develop before the male. Immature, mature and gravid regions are recognizable and, owing to the delayed differentiation of the genitalia, the immature region occupies half of the entire length of the strobila. Once differentiated, however, the female genitalia develop with extreme rapidity and functional female organs are apparent in only two segments (Figs. 1, 6). Anlagen of the female organs first appear in segment 19 but not until segment 27 is reached do they show any differentiation. In segments 28 and 29 they are fully developed and functional while in the 30th segment the ovary and vitellarium have already degenerated. The male genitalia are slower in their development. The anlage of the cirrus-sac appears first in segment 20, but the cirrus-sac is only completely formed in segment 31, where of the female genitalia only the uterus and degenerating receptaculum seminis remain. The testes first appear as undifferentiated masses in segment 27 and reach maturity in segment 32, when the uterus is already distended with eggs. Behind the functional male region, which extends to segment 35, is the gravid region consisting of two segments containing a large lobed uterus, remains of the receptaculum seminis, the cirrus-sac and testes which are beginning to degenerate. The last segment of the complete worm contains only a cirrus-sac, and there is no uterus.



Figs. 1–5. *Andrepigynotaenia haematopodis*, n.g., n.sp.

Fig. 1. Scolex.
Fig. 2. Complete strobila, showing the female genitalia developing before the male.
Fig. 3. Cirrus-sac and invaginated cirrus.
Fig. 4. Cirrus-sac and evaginated cirrus.
Fig. 5. One of the hooks of the cirrus.

In the different parts of the strobila the proglottides vary in outline (Fig. 1). Immediately behind the scolex they are broader than long. Proceeding backwards through the immature region the proglottides become longer than broad. With the onset of female maturity there is an abrupt increase in breadth, a typical segment in this region measuring 0·37 mm. long and 0·88 mm. broad. Posteriorly the proglottides become longer again measuring 1·27 mm. long and 0·83 mm. broad in the gravid region.

The cirrus-sacs alternate irregularly (Fig. 1) and open slightly in front of the middle of the lateral margin of the segment. There is no vagina. The cirrus is extremely powerful, long and armed with hooks (Figs. 4, 5). The powerful nature of the cirrus is doubtless associated with the absence of a vagina, copulation taking place by the penetration of the body by the cirrus.

MUSCULATURE

The superficial musculature of the strobila consists of very delicate circular and longitudinal muscles lying below the basement membrane. The deeper musculature consists of two layers of longitudinal muscles alternating with three layers of transverse (Figs. 9, 15–17). In addition there are dorso-ventral muscles in the medullary parenchyma and radial muscles in the cortical parenchyma.

The outer layer of longitudinal muscles consists of from sixty to seventy bundles arranged regularly in a complete ring. The inner layer consists of about forty bundles which do not form a ring but which are present only dorsally and ventrally. Medially each bundle in both inner and outer layers consists of from ten to twenty-five fibres, laterally the bundles of the outer layer become reduced in size and consist then of only from four to five fibres. The outer layer of transverse muscles is external to the outer layer of longitudinal and consists of numerous fine fibres forming a continuous plate throughout the segment. The middle layer of transverse muscles consists of only a few isolated fibres running transversely between the two layers of longitudinal. The inner layer of transverse muscles is internal to the inner longitudinal layer and the fibres of which it is composed are so few that their existence can only be traced by careful examination.

The dorso-ventral muscles consist of delicate fibres extending through the medullary parenchyma, more particularly in the lateral regions. The radial muscles, too, are delicate fibres radiating through the cortical parenchyma outside the outer transverse layer.

Much of the musculature degenerates towards the posterior part of the strobila. The testes, when fully developed, often extend out between the longitudinal muscle bundles, probably as a result of pressure from the expanding uterus in the centre. The two inner layers of transverse muscles can no longer be recognized in older segments, but the outer thicker layer persists unchanged and acts as a kind of receptacle for the expanding uterus.

EXCRETORY SYSTEM

A dorsal and a ventral excretory vessel extend throughout the strobila on each side. The dorsal vessel measures 0·011 mm. in diameter and the ventral 0·020 mm. The ventral vessel is connected in the posterior region of each segment by a transverse vessel 0·008 mm. in diameter (Figs. 6–8). The transverse vessel is very short, or, in other words, the ventral vessels curve inwards anteriorly and posteriorly in each segment. Both longitudinal vessels pass ventrally to the cirrus-sac (Figs. 6–8, 13, 14). The walls of the excretory vessels are provided with circular muscles, those of the dorsal vessels being considerably better developed than those of the ventral and transverse. Immediately surrounding each of the longitudinal canals is a differentiated layer of parenchyma more densely nucleated than the general parenchyma of the body.

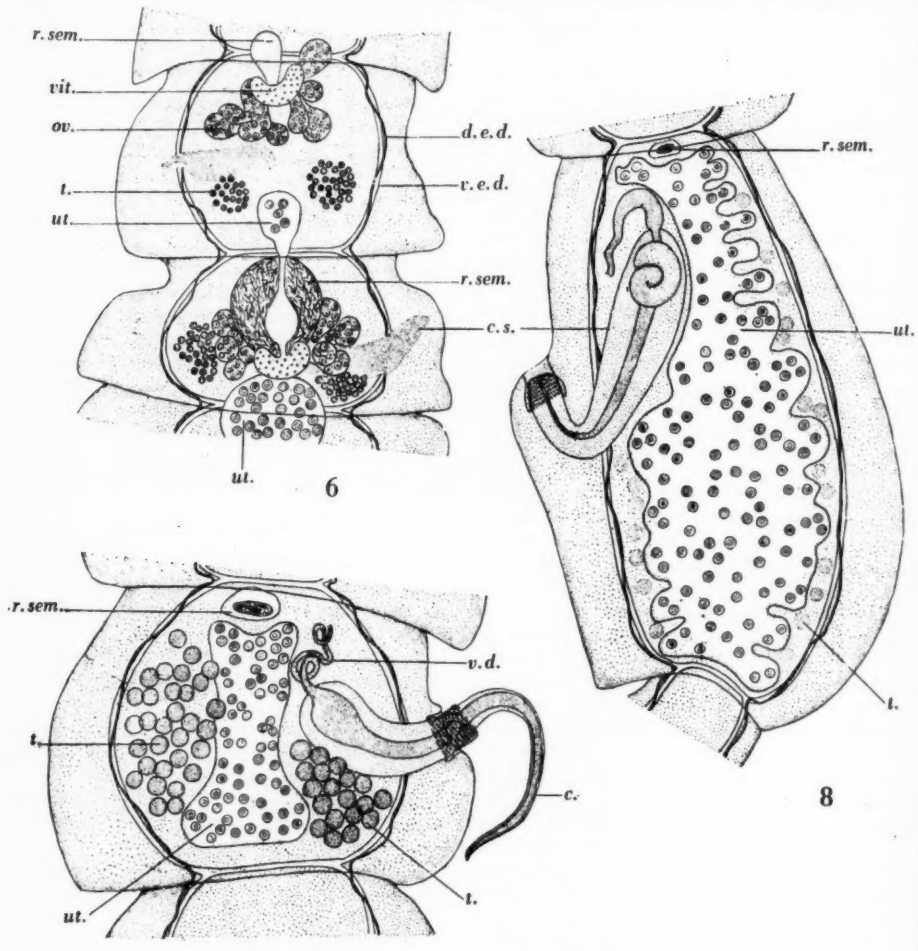
GENITAL SYSTEM

The distribution of the genitalia throughout the strobila has already been discussed. The disposition of the genitalia relative to external segmentation is of special interest. The anlagen of the male organs appear in the middle of the length of each segment (Fig. 1) and the mature testes and cirrus-sac retain this position throughout. The female anlagen, however, are inter-segmental, the ovary and vitellarium of each set of organs occupying the anterior part of one segment while the uterus extends into the posterior half of the segment next in front, later expanding to fill that segment and becoming completely separated from the segment in which it originated (Figs. 1, 6, 17). This explains the absence of a uterus in the last segment of the complete strobila.

Female Genitalia. The female genitalia consist of ovary, receptaculum seminis, vitellarium, shell-glands and uterus. A vagina is absent.

The medially placed ovary is bilobed, each lobe in turn being divided into from four to six clavate follicles (Figs. 6, 11). In transverse section the ovary can be seen to be U-shaped (Fig. 12), the base of the U which is the ovarian commissure being ventral and the arms of the U, or follicles of the ovary, extending dorsally and spreading out through the whole of the medullary field in the dorso-ventral direction. The ovarian follicles measure from 0·045 to 0·068 mm. in diameter. The vitelline gland is kidney-shaped and lies dorsal to

the ovarian commissure (Figs. 6, 11, 12). It measures 0·077 × 0·170 mm. The receptaculum seminis occupies a position between the dorsal vitellarium and the ventral ovarian commissure in diameter. It turns dorsally and is joined by a short spermatic duct from the receptaculum seminis (Figs. 11, 15). In front of the vitellarium the oviduct opens into the ootype, which receives



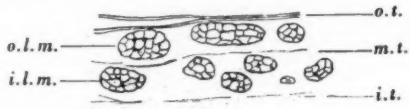
Figs. 6–8. *Andrepigynotaenia haematopodis*, n.g., n.sp.

Fig. 6. Two adjacent proglottides, showing functional female genitalia. Dorsal view.
Fig. 7. Proglottis, showing functional male organs, gravid uterus and remains of receptaculum seminis. Dorsal view.
Fig. 8. Gravid proglottis containing uterus, cirrus-sac, degenerating testes and receptaculum seminis. Dorsal view.

(Figs. 6, 11–13, 15–17). It is a large, distended sac, filled with sperms, and extends from the region of the ovarian commissure to the anterior margin of the proglottis and later into the posterior part of the proglottis next in front. The oviduct arises from the ovarian commissure and measures 0·018 mm. posteriorly a short vitelline duct from the vitellarium and which is surrounded by the shell-glands. From the anterior end of the ootype arises a uterine duct 0·136 mm. in diameter, which runs forwards curving obliquely ventrally in its course to enter the segment next in front, where it expands

into the uterus (Figs. 6, 11). In sagittal section (Figs. 15–17), the early uterus can be seen to proceed antero-ventrally through the medullary parenchyma so that eventually its apex comes to lie ventral to the ovarian commissure of that segment. As development continues, however, and as the ovary and vitellarium degenerate, the uterus extends dorsally through the medullary parenchyma on either side, the two wings eventually uniting dorsally so that these organs become encircled temporarily by the uterus. In its final state the uterus is a large, lobed sac packed with eggs and occupying almost the whole of the medullary

thirty-five testes, while the smaller poral group consists of about twenty, which are confined to the region behind the cirrus-sac (Fig. 7). Each testis is almost spherical in shape and measures 0·050 mm. in diameter. The vasa efferentia from both groups of testes unite dorsally to form a single vas deferens which curves ventrally to enter the cirrus-sac (Fig. 13). The vas deferens is greatly convoluted and packed with sperms. It probably serves as an external vesicula seminalis. The cirrus-sac measures 0·7 mm. in length and has a maximum diameter of 0·196 mm. proximally. At first it is transversely placed in the segment (Figs. 2, 6), but as the seg-



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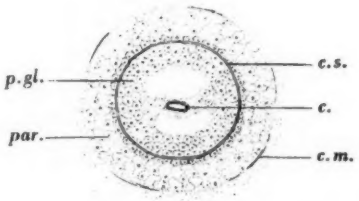

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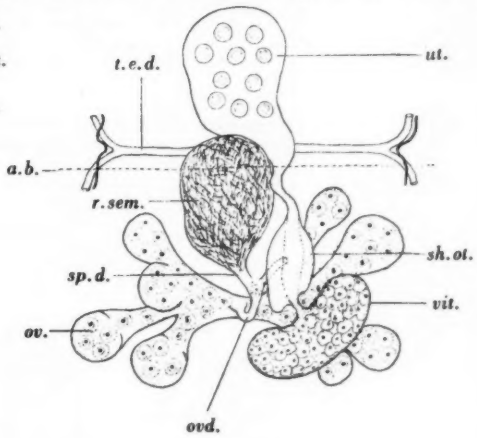

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Figs. 9–11. *Andrepigynotaenia haematopodis*, n.g., n.sp.

Fig. 9. Diagram of portion of musculature in transverse section.
Fig. 10. Transverse section through cirrus-sac.
Fig. 11. Reconstruction of female genitalia; vitellarium displaced slightly to the right. Dorsal view.

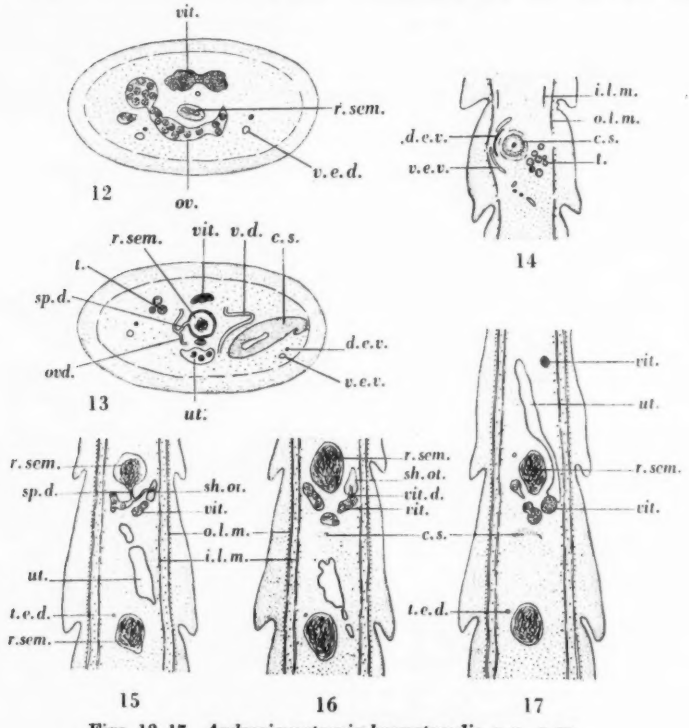
parenchyma except for that region in which the cirrus-sac is situated. The ovary and vitellarium completely disappear, but the receptaculum seminis remains as a vestige at the anterior border of the segment in all segments except the last in the complete worm.

Male Genitalia. In the complete specimen examined the anlagen of the testes first appear in segment 27. Functional testes are present from the 31st to the 35th segments inclusive, after which they begin to degenerate (Fig. 2). Each segment contains from fifty to seventy testes which are arranged in two lateral groups one on either side of the median uterus and internal to the longitudinal excretory ducts of its own side. The aporal group is the larger and more extensive, comprising about

ment grows in length it comes to lie obliquely between the excretory ducts and the uterus (Figs. 1, 8). In a transverse section of the body the cirrus-sac can also be seen to be somewhat obliquely disposed in a dorso-ventral direction (Fig. 13). The proximal end is ventral, and to reach the genital aperture the cirrus-sac passes obliquely upwards dorsally to both lateral excretory ducts (Figs. 13, 14). The wall of the cirrus-sac is composed of a thin layer of circular muscles and the sac is surrounded by an area of dense parenchymatous tissue which is in turn demarcated from the surrounding medullary parenchyma by occasional muscle fibres (Fig. 10). Lining the wall of the cirrus-sac are several layers of cells which seem to be of a glandular nature (Figs. 3, 4, 10). Probably they are prostatic, as Fuhrmann

(1936) suggests for similar cells in the cirrus-sac of *Gynandrotaenia stammeri*. On entering the cirrus-sac the vas deferens enlarges to form an internal vesicula seminalis (Figs. 3, 4). When withdrawn the very long cirrus is coiled up inside the cirrus-sac, towards its proximal end (Fig. 3). On protrusion the portion of the male duct inside the cirrus-sac is straightened (Fig. 4). The cuticle covering what might be termed the apex of the invaginated cirrus is covered with several spirally arranged rows of hooks, and the covering of hooks is continued for some little distance into the invaginated portion (Fig. 3). The hooks measure 0·0114 mm. in length and are of characteristic shape (Fig. 5). As the cirrus evaginates that portion of it covered with hooks is protruded first and this powerfully armed portion, evaginated with force, probably effects the penetration of a segment during copulation. This initial penetration is then followed by complete evagination of the remainder of the cirrus. When the cirrus is fully evaginated (Fig. 4) the hooks can be seen to be restricted to the basal region, the rest of the cirrus being long, tapering and unarmed. It measures 0·88 mm. in length. It seems doubtful whether the cirrus can be withdrawn into the cirrus-sac again after protrusion. The portion of the cuticle armed with spines becomes detached from the rest of the cirrus wall and remains as a collar surrounding the base. It is possible that the prostatic cells inside the cirrus-sac produce a secretion which facilitates the penetration of the body wall or perhaps the wall of the receptaculum seminis. Such a function has been suggested by Fuhrmann (1936) for similar cells in the cirrus-sac of *Gynandrotaenia stammeri*.

Andrepigynotaenia haematopodis belongs to the



Figs. 12–17. ***Andrepigynotaenia haematopodis*, n.g., n.sp.**

Fig. 12. Transverse section through proglottis, showing U-shaped ovary.
Fig. 13. Transverse section through proglottis, showing arrangement of some of female organs and ducts, and developing cirrus passing dorsally to both lateral excretory vessels.
Fig. 14. Sagittal section, showing cirrus-sac dorsal to excretory vessels.
Fig. 15. Sagittal section, showing junction of sperm-duct with oviduct, etc.
Fig. 16. Sagittal section, showing vitellarium, vitelline duct, etc.
Fig. 17. Sagittal section, showing course of uterus from ootype to segment next in front.

family Progynotaeniidae Fuhrmann (1936). Originally the protogynous forms were included in the family Acoleidae. Fuhrmann (1936), however, is of the opinion that, owing to the fact that they all possess certain distinctive characters, in which they differ from the remainder of the Acoleidae, they should be separated from that family and placed in the new family Progynotaeniidae. Fuhrmann states that the strobila in this family consists of only a few segments, from ten to twenty. As *A. haematopodis* of one genus which is characterized by the fact that the male and female organs are in alternate segments. In the family Progynotaeniinae are included the genera *Progynotaenia* Fuhrmann, *Proterogynotaenia* Fuhrmann, *Leptotaenia* Cohn, and now *Andrepigynotaenia* n.g. A comparison of these four genera is given in the following table.

Andrepigynotaenia resembles *Proterogynotaenia* more closely than it does the other two genera of the subfamily. In both the strobila is long and

	Progynotaenia Fuhrmann	*Proterogynotaenia* Fuhrmann	*Andrepigynotaenia* n.g.	*Leptotaenia* Cohn
Scolex	Single crown of hooks	Double crown of hooks	Unarmed?	Single crown of hooks
No. of segments	8–17	20	38	12–15
Musculature	Three longitudinal alternating with two transverse layers (except *P. flaccida* Meggitt (1928))	Two longitudinal alternating with three transverse layers	Two longitudinal alternating with three transverse layers	?
Genitalia	Differentiate rapidly	Differentiate slowly	Differentiate slowly	Differentiate rapidly
Female genitalia	Posterior in proglottis. Uterus may invade small area of proglottis next in front	Inter-segmental	Intersegmental. Receptaculum seminis and uterus enter proglottis next in front. Uterus eventually entirely in proglottis next in front	Segmental. Female behind male
Cirrus-sac	Regularly alternate	Irregularly alternate	Irregularly alternate	Regularly alternate
	Passes ventral to both excretory ducts	Passes between dorsal and ventral excretory ducts	Passes dorsal to both excretory ducts	?
No. of species	6	1	1	1
	Fuhrmann (1909), Nybelin (1914), Baczynska (1914), Meggitt (1928), (2) Skrjabin (1914)	Fuhrmann (1911)	n.g., n.sp.	Cohn (1901)
Host	Charadriiform birds	*Ochthodromus geoffroyi* (Wagl.)	*Haematopus ostralegus* Neumann	*Phoenicopterus roseus* Blanf & Oates
Distribution	Egypt and White Nile	Aru Islands	Britain	Mediterranean

may have as many as thirty-eight segments this diagnostic feature might be modified to 'from 10 to 38 segments'. One other feature in which the present species differs from the other members of the family is in the unarmed nature of the scolex. This feature, however, cannot be definitely established until more material is available, as the hooks in the single scolex obtained may have been lost. Fuhrmann has divided the family into the two subfamilies Progynotaeniinae and Gynandrotaeniinae. The latter subfamily contains only the type species the genitalia slow in differentiating. These two characters are probably interdependent, the length of the strobila presumably being a result of the slow gonadial differentiation. The arrangement of the musculature is similar in both. *Andrepigynotaenia*, however, differs from *Proterogynotaenia* in that the cirrus-sac passes dorsally to both lateral excretory ducts and not between them. It seems that this character may be regarded as one of generic significance, as the arrangement in *Progynotaenia* is again characteristic and distinct from these two.

DEFINITION OF THE GENUS *ANDREPIGYNOTAENIA*

Progynotaeniinae with strobila of about thirty-eight segments and with slowly differentiating genitalia; musculature consisting of two layers of longitudinal muscles alternating with three layers of transverse; female genitalia inter-segmental; uterus when gravid lying entirely in the segment next in front of that to which it belongs; cirrus-sacs irregularly alternating and passing dorsally to both lateral excretory vessels.

Type-species *Andrepigynotaenia haematopodis*, n.sp.

The writers wish to express their indebtedness to the helpful suggestions of the late Professor O. Fuhrmann, and their thanks to Dr H. A. Baylis of the British Museum (Natural History) for the opportunity of studying material of *Progynotaenia* spp.

REFERENCES

Baczynska, H. (1914). *Bull. Soc. neuchâtel Sci. nat.* **40**, 230.

Cohn, L. (1901). *Nova Acta Leop. Carol.* **79**, 171.

Fuhrmann, O. (1909). *Die Cestoden der Vogel des weisses Nile. Results of Swedish Zoological Expedition to Egypt and the White Nile*, 1901, 1909, **27**, 45.

Fuhrmann, O. (1909*a*). *Bull. Soc. neuchâtel Sci. nat.* **36**, 90.

Fuhrmann, O. (1911). *Abh. senckenb. naturf. Ges.* **34**, 259.

Fuhrmann, O. (1936). *Ann. Parasit. hum. comp.* **14**, 3, pp. 261–71.

Fuhrmann, O. (1936*a*). *Rev. suisse Zool.* **43**, 20, pp. 517–18.

Meggitt, F. J. (1928). *Parasitology*, **20**, 315.

Nybelin, O. (1914). *Zool. Bidr. Uppsala*, **3**, 225.

Skrjabin, K. J. (1914). *Zbl. Bakt.* **75**, 79.

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List of abbreviations: *a.b.* anterior border of segment; *a.f.* anlagen of female organs; *a.m.* anlagen of male organs; *c.* cirrus; *c.m.* circular muscle fibres; *c.s.* cirrus-sac; *d.e.d.* dorsal excretory duct; *d.e.v.* dorsal excretory vessel; *g.a.* male genital atrium; *h.s.* sheath of hooks around cirrus; *i.l.m.* inner layer of longitudinal muscles; *i.t.* inner layer of transverse muscles; *m.c.* muscular cone; *m.t.* middle layer of transverse muscles; *o.l.m.* outer layer of longitudinal muscles; *o.t.* outer layer of transverse muscles; *ov.* ovary; *ovd.* oviduct; *p.gl.* prostatic glands; *par.* parenchyma around cirrus-sac; *r.* rostellum; *r.s.* rostellar sac; *r.sem.* receptaculum seminis; *s.* sucker; *sh.ot.* shell-glands surrounding ootype; *sp.d.* spermatic duct; *t.* testes; *t.e.d.* transverse excretory duct; *ut.* uterus; *v.d.* vas deferens; *v.e.d.* ventral excretory duct; *v.e.v.* ventral excretory vessel; *v.s.* vesicula seminalis; *vit.* vitellarium; *vit.d.* vitelline duct.

THE SECOND ECDYSIS OF *TRICHOSTRONGYLUS RETORTAEFORMIS* (ZEDER)

By H. D. CROFTON, B.Sc., Ph.D., *King's College, Newcastle-upon-Tyne*

(With 1 Figure in the Text)

The second ecdysis of *Trichostrongylus retortaeformis* transforms the infective larva into the first parasitic larva, and it is therefore of particular importance in a study of the life-history. A knowledge of the mechanism of this moult is essential to the understanding of the adult parasite. Although several workers have studied exsheathment, their results are conflicting and further work appeared necessary.

Material and methods. Infective larvae of *T. retortaeformis* were obtained from fresh faeces-charcoal cultures, the faeces being obtained from laboratory rabbits in which a pure infestation of *T. retortaeformis* had been established. Larvae were separated from the culture on clean moist filter paper on to which they migrated from the faeces-charcoal mixture. They were counted by a dilution sampling method, samples being taken with a Stoll Pipette.

The experiments were designed to test the effect of saliva, HCl and pepsin, these substances being encountered by infective larvae when introduced into the host's body.

The effect of saliva. Two thousand larvae were placed in each of four Petri dishes. Fresh human saliva was added to two of these dishes and distilled water added to the other two. The four sets of larvae were then incubated at 37° C. After 2, 4, 6, 8, 24, 36 and 48 hr. samples were taken and larvae examined.

No ecdyses occurred during this period but the sheaths of larvae in saliva became thinned and swollen at the anterior end. Bacterial contamination prevented examination after 48 hr.

The effect of different concentrations of HCl. Hydrochloric acid was diluted to give a series of *p*Hs ranging from below 1 to 7. Details are given in Table 1. These *p*Hs were checked very approximately by colour-indicators. 10 ml. of each solution were placed in small Petri dishes and 0·5 ml. of water containing 2000 larvae was added to each. The dishes were incubated at 37° C. for 4 days. A sample was taken after 12 hr. and examined for exsheathed larvae; further samples were taken every 6 hr. The results are given in Table 1.

The table shows that no ecdysis occurred in HCl, but the sheaths were dissolved when the *p*H was 1 or below. At *p*H 2 and *p*H 3 the sheaths did not dissolve but underwent a change so that the surface became 'sticky'. Lapage (1935) describes sheaths which became 'sticky' in HCl. Stephenson (1945) describes the cuticle of *Rhabditis terristris* as 'sticky' after immersion in acid solutions (*p*H 0·5–3·5).

Table 1. *Different p*Hs *of* HCl

Time in hours	*p*H: Less than 1 L	S	1 L	S	2 L	S	3 L	S	4, 5, 6, 7 L	S
0–12	D	st	A	sw	A	U	A	U	A	U
12–18	D	d	A	st	A	U	A	U	A	U
18–24	D	d	D	st	A	st	A	U	A	U
24–36	D	d	D	st	A	st	A	st	A	U
36–96	D	d	D	d	A	st	A	st	A	U

L = larvae; S = sheath; A = alive; D = dead; st = 'sticky'; sw = swollen; d = dissolved; U = unchanged

The effect of HCl *and pepsin.* Hydrochloric acid was diluted to make solutions of *p*H 1·1, 2·68, 3·67, 4·10, 4·32, 4·50, 4·67, 4·85, 5·10, 5·50, 5·85, 6·0, 6·85, 7·0. These were checked using a quinhydrone electrode. Two Petri dishes were used for each *p*H; to one series pepsin solution was added to give a final pepsin concentration of 0·2%. 2500 larvae were placed in each dish and all the dishes incubated at 37° C. After 48 hr. the larvae in each dish were examined, a dilution sampling method being used as before. The number of living and dead larvae which had exsheathed were noted (Fig. 1). Clearly there were no more moults in the HCl solutions than in ordinary cultures; where pepsin had been added, however, a high percentage of moults occurred in *p*Hs 1·1–5·1. It is interesting to compare this curve with one representing the activity of pepsin in different *p*Hs (substrate haemoglobin) (see Northrop 1939). Considering the difference in substrate the resemblance is close.

The effect of HCl *and boiled pepsin.* Previous experiments showed that the addition of pepsin to HCl brought about exsheathment of the larvae placed in the acid. To determine whether the effect of adding pepsin was due to pepsin being a proteolytic enzyme the following experiment was made:

Boiled pepsin solution was added to dilute HCl to

give two solutions of *p*H 1·1 and 4·54, each containing 0·2 % boiled pepsin. Two other solutions of HCl were prepared to give *p*Hs of 1·20 and 4·60 and to contain 0·2 % fresh pepsin. 5000 larvae were placed in each of the four solutions and these were incubated as before. Larvae were examined at intervals, the same sampling method being used as in the previous experiments. The results of this

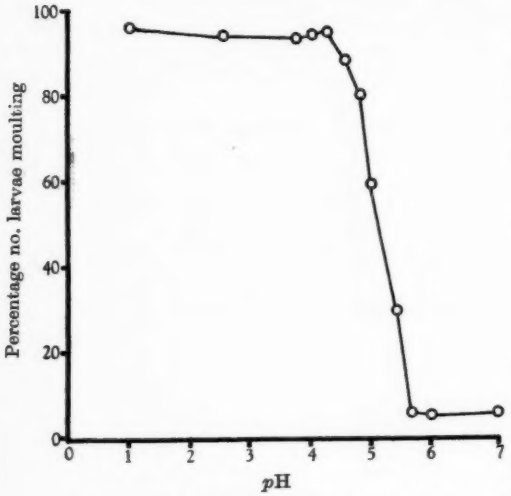

Fig. 1. Percentage of larvae moulting in pepsin at different *p*Hs.

experiment given in Table 2 show that while unboiled pepsin and HCl induced exsheathment of larvae, boiled pepsin and HCl did not. This suggests that ecdysis occurred as the result of the proteolytic

Table 2.

Time in hours	% larvae moulting			
	Boiled pepsin		Unboiled pepsin	
	*p*H 1·1	*p*H 4·54	*p*H 1·20	*p*H 4·60
0	0	0	0	0
2	0	0	5	0
3	0	0	7	5
24	3	0	90	45
48	5	1	95	85
60	5	4	95	90
72	5	7	95	90
96	5	9	95	90

action of the pepsin; in this case the action might be expected to be more rapid in lower *p*H. The table shows that ecdysis occurred in 90 % of the larvae in 24 hr. at *p*H 1·20, whereas moulting of 90 % of the larvae was not recorded until 60 hr. at *p*H 4·60.

DISCUSSION

The experiments showed that pepsin induced exsheathment of larvae, which is in agreement with the observation of Looss (1911), who states that larvae of *Ancylostoma* sp. moulted in gastric juice. The work of Goodey (1922) appears to contradict this, but as no mention of temperature or *p*H is made it may be that the pepsin was not used in the presence of acid and/or the larvae were not incubated. Lapage (1935) describes experiments in which larvae were incubated at 38° C. in an HCl extract of the scrapings of a calf's stomach. He recorded some moults after 9 days. HCl was found to be ineffective in producing ecdysis, but at low *p*H the sheath was dissolved. Veglia (1915), Theiler & Robertson (1915) and Mönnig (1930) state that moults occurred in HCl. It seems probable that only a small number of moults occurred in the experiments of the above workers, and a small number of exsheathments occur in normal cultures.

Table 1 and Fig. 1 show that in HCl below *p*H 4 larvae were either killed or their sheaths altered without ecdysis occurring, and, as might be deduced from a pepsin *p*H-activity curve (Sorenson, 1909), that few ecdyses occur above *p*H 5. The optimum *p*H for survival and ecdysis might be expected, therefore, to lie between 4 and 5. Redman, Willemot & Wokes (1927) state that the *p*H of the stomach of rabbits is approximately the same as that of guinea pigs and give figures for guinea pigs which range from 4·0–5·2.

Threlkeld (1933), working with *Ostertagia circumcincta*, showed that ecdysis in the sheep's stomach did not occur until the third day after the larvae had been swallowed. Table 2 shows that at *p*H 4·68 60 hr. elapsed before 90 % of the larvae had moulted. It seems probable, therefore, that ecdysis induced by HCl and pepsin is the method normally occurring in the rabbit's stomach. Ecdysis thus induced occurs by the loss of an anterior cap of the sheath; this Lapage (1935) admits is the normal method, although when he used 'Milton' (a dilute Na hypochlorite solution) the larvae emerged by a rent in the sheath in the oesophageal region. It is not known why the action of pepsin should induce the loss of the anterior cap, but it seems probable that there is a difference in the nature of the cuticle in this region.

SUMMARY

(1) Ecdysis of the infective larvae of *Trichostrongylus retortaeformis* was induced by the action of pepsin.

(2) Exsheathment occurred by the loss of the anterior cap.

(3) *p*H 4–5 is shown to be the most favourable *p*H for ecdysis and survival. This *p*H is within the range quoted for the stomach contents of rabbits.

(4) Ecdysis was complete in the majority of larvae after 60 hr.

I am grateful to Professor A. D. Hobson for advice and criticism and to Mr W. Lyle-Stewart for encouragement and for providing facilities for this work.

REFERENCES

GOODEY, T. (1922). *Ann. Appl. Biol.* **9**, 33.

LAPAGE, G. (1935). *Rep. Dir. Inst. Animal Path. Univ. Camb.* **4**, 280.

LOOSS, A. (1911). *Rec. Egypt. Govt. School Med. Cairo*, **4**, 159.

MÖNNIG, H. O. (1930). *Rep. Dir. Vet. Serv. Anim. Indust. Dep. Agric. Union S. Afr.* **16**, 175.

NORTHROP, J. H. (1939). *Crystalline Enzymes.* Columbia Univ. Press.

REDMAN, T., WILLEMOT, S. G. & WOKES, F. (1927). *Biochem. J.* **21** (1), 589.

SORENSON, S. P. L. (1909). *Biochem. Z.* **21**, 131.

STEPHENSON, W. (1945). *Parasitology*, **36** (3, 4), 158.

THEILER, A. & ROBERTSON, W. (1915). *Rep. Dir. Vet. Serv. Anim. Indust. Dep. Agric. Union S. Afr.* **3**, **4**, 293.

THRELKELD, W. L. (1933). *Tech. Bull. Va Agric. Exp. Sta.* 52.

VEGLIA, F. (1915). *Rep. Dir. Vet. Serv. Anim. Indust. Dep. Agric. Union S. Afr.* **3**, **4**, 349.

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A NEW GENUS OF NEMATODES, PARASITIC IN THE PIKA

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(With 11 Figures in the Text)

Four specimens of the pika (*Ochotona rufescens* Gray) were captured, and on dissection three of them were found to be heavily infested with a Nematode worm in the large intestine.

The worm belongs to the family Oxyuridae Cobbold, 1864, and the subfamily Oxyurinae Hall, 1916. It resembles closely the genus *Dermatoxys* Schneider, 1866, in having three lips, each with two papillae, cervical alae, a short vestibule armed with three teeth, a longitudinal row of crests in front of the cloaca in the male, a long tail in the female, the vulva in front of the middle of the body, and asymmetrical eggs with a plug at one pole. It differs, however, from this genus in that the crests in the male are oval and not transverse, and that the caudal alae do not reach the tail-tip There is a large median cushion-like papilla situated behind the cloacal aperture or just before the last pair of postanal papillae. The species is, however, most strikingly characterized by the remarkable cephalic appendages, in which respect it resembles to some extent two other genera, viz. *Acanthoxyurus* Sandground, 1928 and *Syphacia* Seurat, 1916. In the genus *Acanthoxyurus* the cephalic appendages are in the form of one or two pairs of backwardly-directed subdorsal 'chitinized spines' (Sandground) or 'tusks' (Monnig) projecting freely from the 'frill', while in *Syphacia obubra* Baylis, 1936, they are in the shape of two thickened horseshoe-shaped folds in the cuticle of the frill. In all these cases the structures are described in females only. In the present specimens the cuticle of the head region, in both sexes, is expanded and projects freely to form appendages in the form of a pair of dorsal cephalic wings or shields. As it is thus clearly differentiated from *Dermatoxys*, *Acanthoxyurus* and *Syphacia*, it is proposed to erect a new genus *Cephaluris* for the reception of the species.

Cephaluris, n.g.

Medium-sized worms. Mouth surrounded by three bilobed lips, each bearing a pair of papillae. Cervical alae present. Head with a pair of cuticular, dorsal and backwardly-directed cephalic shields in both sexes. Vestibule short and armed with three teeth. Oesophagus followed by a bulb, separated from the rest by a constriction. *Male:* Tail long. Caudal alae not reaching the tip. A long curving ventral row of oval crests in front of cloaca. A number of caudal papillae close to anus, and a large median cushion-like papilla situated before the last pair of postanal papillae. Spicule short, lightly chitinized. Accessory piece absent. *Female:* Tail long, gradually tapering to a point. Vulva in front of the middle of the body. Oviparous. Eggs asymmetrical, with rugose thick shells having a plug at one pole.

Genotype.—*Cephaluris ochotonae* n.sp.

Cephaluris ochotonae, n.sp. Medium-sized worms with cuticle of the body finely striated transversely. The cuticle of the head region first extends a little forward as a circum-oral membrane supported by four cephalic papillae, two dorsal and two ventral, and then behind the dorsal papillae it is expanded and projects dorso-posteriorly to form a pair of cuticular dorsal cephalic shields. Broad cervical alae are present and extend to the level of the anterior or posterior end of the oesophageal bulb, from which level lateral alae run posteriorly to the level of the anus. The mouth is more or less circular, with three small lips, each bearing two lateral papillae. The vestibule is short and armed with three teeth, surrounding the opening of the oesophagus. The oesophagus is thickened at both ends and has a posterior bulb separated from the rest by a constriction.

The male is about 8·27 mm. long and 0·23 mm. thick. The head is about 0·08 mm. in diameter and the dorso-cephalic shields are 0·07 mm. long. The cervical alae are about 0·33 mm. long and 0·035 mm. wide. The lateral alae are 0·051 mm. wide. The vestibule is 0·008 mm., and the oesophagus, without the bulb, 0·42 mm. long, the size of the bulb being 0·125 × 0·106 mm. The nerve-ring is situated 0·22 mm. from the anterior end of the body.

The tail is 0·67 mm. long and bent ventrally behind the cloaca, from which level it narrows gradually to a point. A long curving ventral row of small longitudinal oval crests is present. It begins about 0·44 mm. in front of the cloaca and runs forward to a distance of 1·29 mm. The caudal alae are 0·33 mm. long and do not reach the apex. The caudal papillae are grouped mainly around the cloaca, where the cuticle is prominent. There are sixteen paired and one unpaired caudal papillae, seven pairs of which are pericloacal. Of these seven pairs two pairs are preanal, one pair adanal, and four pairs postanal. The unpaired papilla is large, pedunculate and cushion-like, and is situated

Fig.
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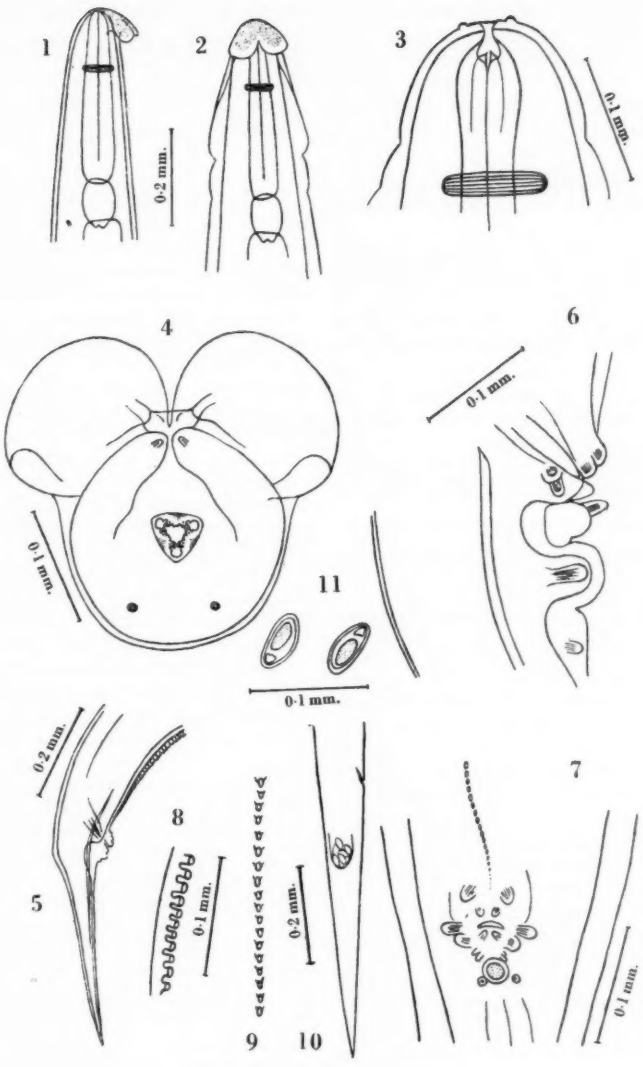

Figs. 1–11. *Cephaluris ochotonae*

Fig. 1. Anterior extremity of female, lateral view.
Fig. 2. Anterior extremity of female, dorsal view.
Fig. 3. Anterior extremity of female, ventral view.
Fig. 4. Anterior extremity of female, end-on view.
Fig. 5. Posterior extremity of male, lateral view.
Fig. 6. Cloacal region of male, lateral view.
Fig. 7. Cloacal region, ventral view.
Fig. 8. Preanal row of crests, lateral view.
Fig. 9. Preanal row of crests, ventral view.
Fig. 10. Posterior extremity of female, lateral view.
Fig. 11. Eggs.

medially behind the pericloacal and just in front of a posterior pair of papillae. The spicule is short and very lightly chitinized, its length being about 0·089 mm. A gubernaculum is absent.

The female is about 18·45 mm. long and 0·68 mm. thick. The head is 0·23 mm. in diameter and the dorsal cephalic shields are 0·09 mm. long. The lateral alae are 0·06 mm. wide. The vestibule is 0·012 mm. and the oesophagus, without the bulb, 0·59 mm. long. The bulb measures 0·17 × 0·16 mm. The nerve-ring is situated at 0·29 mm. from the anterior end of the body.

The tail is long and gradually tapers to a point, its length being 3·56 mm. The vulva is prominent, situated in front of the middle of the body, its distance being about 8·1 mm. from the anterior end. It is covered by a short flap on its anterior lip. The vagina is short, curving posteriorly from the vulva. The ovaries are parallel. The uterine tubes extend posteriorly beyond the anus. The eggs measure 0·084 × 0·044 mm. They are asymmetrical, with thick shells, stippled externally and with a plug at one pole.

Type-specimens. No. W 3468/1, Zoological Survey of India (Ind. Mus.), Calcutta.

I wish to acknowledge my indebtedness to Dr H. A. Baylis, British Museum (Natural History), London, for his valuable suggestions and for kindly sending me the description of his species *Syphacia obubra*. I have also to express my thanks to Dr Sunder Lal Hora, Department of Fisheries, Bengal, for his constant help in different ways.

REFERENCES

Baylis, H. A. (1936). Some parasitic worms from the British Cameroons. *Ann. Mag. Nat. Hist.* (10), **17**, 257.

Baylis, H. A. & Daubney, R. (1926). *A Synopsis of the Families and Genera of Nematoda.* London: British Museum (Nat. Hist.).

Yorke, W. & Maplestone, P. A. (1926). *The Nematode Parasites of Vertebrates.* London.

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NOTE ON A CILIATE PROTOZOON, PROBABLY *GLAUCOMA PYRIFORMIS*, PARASITIC IN CULICINE MOSQUITO LARVAE

By J. MUSPRATT, *Department of Entomology, South African Institute for Medical Research, Johannesburg*

INTRODUCTION

Two unidentified types of ciliate parasites were previously found (Muspratt, 1945) in the body-cavity of several species of tree-hole breeding culicine mosquito larvae at Livingstone in Northern Rhodesia. It now seems certain that these two forms, referred to as types *A* and *B*, belong to the same organism. The type *A* is that which multiplies rapidly by binary fission in the haemocoel of the larva finally causing its death, whereas the type *B* divides seldom or not at all and does not kill the larva.

Miss V. Lewis of the Department of Zoology at the University of Cape Town kindly undertook the staining and examination of fixed smears containing the liberated parasitic forms which were sent to her. These were fixed in Schaudinn's solution and were accordingly stained with Heidenhain's haematoxylin, so as to show the nuclear structure, and in one case counterstained with fast green. Owing to war-time shortage of reagents it was not possible to prepare other specimens specially fixed and stained to show the arrangement of the cilia, but nevertheless this can be seen fairly clearly in some of the preparations.

I have been informed by Miss Lewis (*in litt.*) that the nuclear structure of the type *A* appears to be identical with the illustrations by Keilin (1921) of *Lambornella stegomyiae*, but she is inclined to agree with the suggestion of Wenyon (1926) that this ciliate is actually *Glaucoma pyriformis* Ehrenberg. Examination, by an expert, of the structure and position of the cytostome in the living ciliates would be the only way to arrive at a definite decision. Unfortunately, this examination could not be made as attempts to mail the living ciliates from Livingstone to Cape Town were unsuccessful.

The nuclear structure of the type *B* seems to be the same as type *A*, but the one or two preparations of the former are not perfect and therefore not very easily compared in detail.

The type B, *an encysting stage?*

From a laboratory study of the living organisms the writer latterly came to the definite conclusion that the two types were different forms of the same ciliate and, as stated above, this is borne out by an examination of the stained specimens. In certain culicine larvae it seems that there is a tendency for the slow-reproducing, or non-reproducing, type *B* to arise rather than the fast-multiplying type *A*. Miss Lewis has suggested (*in litt.*) that the type *B* is the organism in a pre-encystment stage. Thick-walled or other cysts have not been seen in the body-cavity of larvae containing this form, but the writer has recorded (1945) dissecting out the type *B*, still alive, from the adult mosquito; so it is possible that it encysts in the adult and it may be in this way that the parasite is carried from one tree-hole to another. There is reason to suppose, however, that it is perhaps a degenerate form due to an unsuitable species of culicine host (see below).

LABORATORY OBSERVATIONS

1. *Transmission of the type* A *from one culicine larva to another*

A. *Escape from the host.* Certain facts emerge from a study of the parasite in the laboratory: (*a*) The ciliates must escape from the mosquito larva before it dies as those which remain do not survive for very long in the dead larva. (*b*) As suggested by Lamborn (1921) escape from the living larva is usually effected as a result of the rupture of one or more of the anal papillae. This occurs at a time when the body fluid of the larva has apparently become so thin due to parasitization, that the change in the concentration between it and the water does not affect the escaping organisms and cause them to swell up and burst, as happens if they are released artifically in the early stages of parasitization. (*c*) Rupture of the anal papillae does not occur with all species of culicine larvae even when they are packed full of the ciliates. The larval host on which the *Glaucoma* is dependent at Livingstone would appear to be *Aëdes* (*Finlaya*) *fulgens* Edw. In this species rupture of at least one of the anal papillae occurs in nine out of ten of those larvae which contain large numbers of the type *A* form.

B. *The cuticular cysts and penetration of the cuticle.* Although no ciliates in pure culture were obtained it can be stated with absolute confidence that the free-living organisms encyst on the cuticle of the larvae, usually just after ecdysis when perhaps the

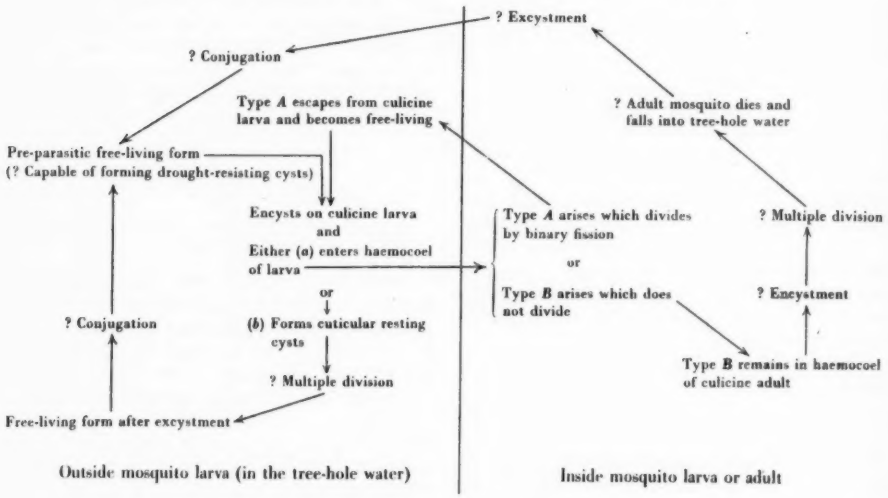
new cuticle is soft; they then pass through it into the haemocoel, unless the cuticle becomes too thick owing to the growth of the larvae or shedding of the pelt takes place during the next moult, before the encysted organisms have been able to pass through. But the question as to whether the free-living ciliates may also be swallowed by the larvae and pass through the wall of the gut has not been investigated.

Contrary to the findings of Lamborn (1921) the writer has obtained infection of laboratory bred larvae within a few days following the release of the ciliates from the host. Large numbers of the ciliates were collected in test tubes when escaping via the ruptured anal papillae of *A. fulgens* larvae and small

to penetrate owing to thickening of the cuticle or shedding of the pelt, the following has been observed: the vacuole stopped contracting and a smaller brownish coloured resting cyst was formed inside the original cyst. These resting cysts were kept in rain water and some of the organisms excysted a few days later; but it is not known what the resultant form looks like and as the above has been seen on only one or two occasions it requires confirmation.

It has not been determined how the cuticular cysts are formed; the free-living organisms are mostly very much larger than the cysts they produce. The ciliates are usually found in tree-holes in which there is a scarcity of bacterial food in the

Diagram of possible life-cycle of the ciliate (unknown parts marked with a query)


? Excystment
? Conjugation
Type A escapes from culicine larva and becomes free-living
? Adult mosquito dies and falls into tree-hole water
Pre-parasitic free-living form
(? Capable of forming drought-resisting cysts)
Encysts on culicine larva and
Either (a) enters haemocoel of larva
or
(b) Forms cuticular resting cysts
? Multiple division
Free-living form after excystment
? Conjugation
Type A arises which divides by binary fission
or
Type B arises which does not divide
? Multiple division
? Encystment
Type B remains in haemocoel of culicine adult
Outside mosquito larva (in the tree-hole water)
Inside mosquito larva or adult

laboratory-bred larvae of *A. aegypti* which were put into these test tubes became covered with cysts: the organisms appearing in the haemocoel within 24 hr. after this. There were very few other protozoan organisms present, except in some cases a few vorticellids and rotifers, which were easily recognized.

The actual penetration of the cuticle by the organism has not been studied in detail on a living larva, as this would be rather difficult, but the cuticular cysts and stages in the penetration process were studied on pelts of second and third stage larvae just after ecdysis when some of the encysted organisms were partly through the cuticle. The original hemispherical cyst is not a rigid structure and there is a contractile vacuole on the top which discharges into the water. On one or two occasions when the encysted ciliate has been unable

water containing the culicine larvae, so it may be that in between each moult the semi-starved larvae take longer than usual to form a thick cuticle; this perhaps enables the parasite, which, as stated above, normally encysts on the cuticle just after ecdysis, to pass through more easily.

2. *Infection of laboratory-bred larvae of* Aëdes (Stegomyia) aegypti *L.*

Owing to the ease with which this species of mosquito is bred in captivity, larvae of *A. aegypti* were used for infection experiments. With this species the anal papillae of infected larvae do not often rupture of their own accord, but when an advanced stage of parasitization has been reached the protozoa may be released by amputation of the papillae. Ciliates released in this way will encyst on the cuticle of other larvae and, subject to the

conditions mentioned above, will penetrate into the haemocoel. It has been found, however, that after a few infections effected by this means the *Glaucoma* strain begins to degenerate and, either only type *B* forms arise in the blood-fluid or, the organisms fail to develop after passing through the cuticle. Therefore *Aëdes aegypti* must be regarded as an unsuitable host of the parasite. Unfortunately, attempts to breed *A. fulgens* in the comparatively small cages available were unsuccessful—no fertile eggs being obtained.

3. *Experiments with anopheline larvae*

In experiments with larvae of *A. gambiae* and *A. funestus*, although the organisms were seen to encyst on the cuticle, in only one instance did any survive for long in the haemocoel. In the case of this larva, of *A. funestus*, the one or two which entered did not reproduce, but a single type *A* organism lived for some days.

DISCUSSION

It is unfortunate that the question as to the identity of *Lambornella stegomyiae* and *Glaucoma pyriformis* could not have been settled by a study of the Livingstone material, as it seems likely that the assumption of Wenyon (1926) that they are morphologically the same is correct. *G. pyriformis*, however, has not been previously regarded as naturally adapted to a specific parasitic mode of life, although Janda & Jírovec (1937) have demonstrated that organisms from a purely free-living strain when injected into certain insects readily become falcultative parasites. Therefore it seems possible that there may be two biological varieties of this protozoan.

Although, as stated, the free-living ciliates of the Livingstone material usually seek to encyst on small larvae immediately after the larvae have moulted; if only fully-grown larvae are present many of the organisms will encyst on the thick cuticle of these in spite of the fact that they are seldom able to penetrate into the haemocoel, except possibly those which have adhered to the anal papillae. This accounts for the cysts on Lamborn's material (Keilin, 1921) which caused confusion as to the primary object of the attachment to the larval host. It seems therefore possible that the cuticular resting cysts formed on a larva when the ciliate has been unable to penetrate into the haemocoel may lie dormant for any time from a few days to a few months. These resting cysts would of course be left behind in the water of the tree-hole on the pelt of the larva after pupation. Possibly changes in the concentration of the water stimulate excystment of the ciliate, as dilution by rain would correspond to the hatching out of more culicine larvae from eggs deposited on the sides of the tree-hole and left high and dry, which is the usual egg-laying habit of tree-hole breeding *Aëdes*.

The above suggestion will account for the time lag in infection observed by Lamborn in his experiments, but in all probability resting cysts capable of withstanding long periods of desiccation are also formed by the free-living ciliates when the tree-holes dry up for some months during the rainless winter season, as is the regular course of events at Livingstone.

One difference in the parasitic habit from the *Glaucoma pyriformis* studied by MacArthur (1922) is that the ciliates do not attack the eyes of the larval host, but in the case of very small larvae, when they first enter they tend to congregate around the heart. A suggestion as to the possible life-cycle of the ciliate is given herewith in diagrammatic form based on analogy with the life-cycles of certain other parasitic holotrichous ciliates.

SUMMARY

1. Stained specimens of two forms of a ciliate parasite resembling *Lambornella stegomyiae* Keilin which is probably *Glaucoma pyriformis* Ehrenberg are discussed. These were collected from the haemocoel of culicine mosquito larvae at Livingstone, Northern Rhodesia. The form previously referred to as type *A* (Muspratt, 1945) is the fast multiplying form which destroys the larva and that referred to as type *B* is considered to be either an encysting or degenerate form.

2. In nature the larva of *Aëdes* (*Finlaya*) *fulgens* Edw. is the most suitable host of the parasite because the normal (type *A*) parasitic ciliates are dependent on escaping from the larval host before death by the rupture of the anal papillae. This occurs most frequently in parasitized larvae of the above species.

3. The encystment of the organisms on the cuticle of the larvae is discussed with reference to statements by previous workers. Stages in the penetration of the cuticle by the organisms and the formation of cuticular resting cysts have been observed.

4. Larvae of *A. aegypti* used in laboratory experiments proved to be unsuitable as: (*a*) The anal papillae do not rupture easily. (*b*) After some time the reproductive power of the ciliates degenerates if they are transmitted from larva to larva of this species only.

5. Attempts to destroy anopheline larvae with the parasite were negative although the ciliates entered the haemocoel.

6. A suggestion as to the possible life-cycle is appended in the form of a diagram.

I am indebted to Miss Lewis for staining and examining the fixed smears sent to her at the Department of Zoology of Cape Town University and to Dr Botha De Meillon of the South African Institute for Medical Research, Johannesburg, for advice on several questions in connexion with this work.

REFERENCES

JANDA, V. & JÍROVEC, O. (1937). *Mém. soc. zool. tchéc.* (*Prague*), **5**, 34. Cited in *Zool. Ber.* (1938), **46**, 85.
KEILIN, D. (1921). *Parasitology*, **13**, 216.
LAMBORN, W. A. (1921). *Parasitology*, **13**, 213.
MACARTHUR, W. P. (1922). *J.R. Army Med. Cps*, **38**, 83.
MUSPRATT, J. (1945). *J. Ent. Soc. S. Afr.* **8**, 13.
WENYON, C. M. (1926). *Protozoology*, **2**, 1183. London: Baillière, Tindall and Cox.

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